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13. ABSTRACT (Maximum 200 Words)

Proton Magnetic Resonance Spectroscopy (MRS) in conjunction with Statistical Computerised Strategies (SCS) provides an alternative modality for the pathological diagnosis and prognosis of breast fine needle aspiration (FNA) biopsies. Benign breast lesions are distinguished from invasive cancer with a sensitivity and specificity of 95% and 96%, respectively, when visual inspection methods alone are employed. These spectra all had a signal to noise ratio (SNR) >25. Ductal carcinoma *in situ* (DCIS) specimens were ranked by MRS as benign or malignant. This ranking is not explained by histopathology, suggesting MRS is reporting on chemical differences which are not morphologically manifest.

Analysis of MRS data using SCS distinguishes benign and malignant lesions with a sensitivity and specificity of 92% and an accuracy of 93% for all spectra. MRS combined with SCS also reports on vascular invasion and lymph node involvement for patients with invasive breast lesions with an accuracy of 92% and 95%, respectively.

A multicentre trial is now underway to verify the MRS method in four international sites: the Karolinska Institute, Stockholm, Sweden; Beth Israel and Deaconess Hospital, Boston, USA; North Western University, Chicago, USA; Royal Adelaide Hospital, Adelaide, Australia.

Proton MRS on FNA from axillary nodes can detect the presence of metastatic breast disease. The method is now being used to assess sentinel lymph nodes.

Two dimensional (2D) COSY MRS was unable to discriminate benign and malignant pathologies in breast tissues due large lipid contribution in the spectra or in breast FNA due to inadequate signal to noise.

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FOREWORD

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INTRODUCTION

Recent improvement in breast cancer patient outcome is due to early diagnosis and effective management. The triage of mammography, clinical examination and fine needle aspiration biopsy is currently used to identify early breast cancers. Magnetic resonance imaging (MRI) has now been added to select women with breast abnormalities requiring biopsy. However, mammography and MRI are unable to distinguish cellular changes which correlate with the development of cancer and are unable to predict patient outcome. Fine needle aspiration cytology following mammography has reduced the incidence of unnecessary surgery but cannot predict at-risk status or tumour progression.

A new technology which could report on alterations to cellular chemistry and provide an adjunct to cytology would offer both independent and objective assessment of breast tissue. The potential then exists to identify an immediate or more distant predisposition towards breast cancer and thus screen, monitor and offer management protocols aimed at reducing tumour risk. Magnetic resonance spectroscopy offers this diagnostic modality and has been successfully developed to monitor tumour development and progression in other organs.

In the clinical situation where an established diagnosis of breast cancer has already been made, current predictors of patient outcome are of limited value. Despite methods of evaluation such as tumour typing and grading, surgico-histopathological staging, hormone and growth factor receptor status, genetic markers and ploidy, the reason for the variable clinical outcome in patients with established widespread metastatic disease remains unknown. The identification of biochemical markers both within primary and metastatic tumour deposits may be of great value in identifying patients who are likely to respond to specific therapeutic regimes.

This proposal is aimed at developing magnetic resonance spectroscopy to be used as an adjunct to diagnostic histopathology. The MRS method offers the possibility of earlier diagnosis of cellular abnormalities in predisposed women, a precise documentation of the biological abnormalities of the cells, conclusive predictive value, clear distinction between cellular atypia, progressive and non-progressive carcinoma *in situ* and invasive cancer and the potential to determine patient outcome. These advantages over current technology warrant the thorough investigation of the application of magnetic resonance spectroscopy to breast pathology.

SUMMARY OF PROGRESS to April 28, 1997

To the end of 1997 218 FNA have been obtained from patients undergoing breast surgery and analysed by proton MR spectroscopy at 360 MHZ (8.5 Tesla) and results compared with histopathological analysis. The results of blinded MR analysis were compared with currently employed diagnostic approaches used in assessment of breast lesions. Invasive carcinoma was identified spectroscopically by an increased signal at 3.25 parts per million (ppm) attributable to choline-containing metabolites. Discrimination between invasive carcinoma (n=82) and normal or benign (n=106) or carcinoma *in situ* (n=17) was made based on the intensity of the 3.25 ppm resonance standardised to the resonance intensity at 3.05 ppm ($p < 0.0001$, Mann-Whitney U test). An MRS ratio of < 1.7 was recorded for 102 out of 106 normal or benign lesions. All carcinoma-*in-situ* samples with comedonecrosis or a microinvasive component (n=6) were ranked by MRS with invasive carcinoma while those with *in-situ* disease alone ranked with benign (n=11). The sensitivity and specificity of MRS on FNB for benign versus invasive breast cancer was 95% and 96% respectively

SUMMARY OF PROGRESS from April 29, 1997 to April 28, 1998

Publications:

1. Mackinnon WB, Barry PA, Malycha PL, Gillett DJ, Russell P, Lean, CL, Doran, ST, Barraclough BH, Bilous M, Mountford CE, FNB specimens of benign breast lesions distinguished from invasive cancer *ex vivo* with proton MRS, *Radiology*, 204:66, 1997.
2. Lo W, Lean CL, Dowd S, Malycha PL, Russell P and Mountford CE, Fine-needle biopsy of human lymph nodes. *Proc. Int. Soc. Mag. Res. Med.*, 1: 327, 1998.
3. Mountford CE, Breast Cancer and MRS, *Life Sciences*, Vol 10 (5) p 22, 1998.
4. Malycha PL and Mountford CE, *Australian Journal of Surgery*, In Press, 1998.

Experimental Progress:

Increase in Number of Breast FNA Biopsies

A further 68 FNA biopsies have been examined by 1 Dimensional MRS to determine the effect of A) storage time and B) method of collection (*ex vivo* or *in vivo*) on the MRS correlation with pathology.

A) Effect of Time Stored at -70°C

Experiments are underway to determine if time in storage at -70°C affects the MRS fingerprint in such a way as to invalidate the MRS correlation with pathology. Both benign and malignant specimens degrade in long term storage and this may result in the chemical markers becoming unreliable. Data shown in Figure 1 indicate that only specimens under six months are amenable to analysis.

B) Effect of Collection Method

Comparison of MR spectra of FNA biopsies obtained *in vivo* (prior to surgical excision) and *ex vivo* (following excision of the lesion) are underway (Figure 2). Preliminary data indicate that FNA obtained *in vivo* generate MR spectra equal in signal to noise and spectral resolution to *ex vivo* FNA spectra and thus amenable to visual and multivariate analysis. It has yet to be determined if the resonance ratio discriminating the two categories remains the same.

Multivariate Analysis of One Dimensional MR Data

Robust multivariate analysis has commenced in collaboration with the National Research Council of Canada. Initial classification of 55 benign and 69 malignant samples gave an accuracy of 90%. This will be improved by increasing the numbers in each pathology to at least 100 per class.

Two Dimensional (2D) MR Assessment of Breast Tissue and FNA Biopsies

Two dimensional (2D) Correlated Spectroscopy (COSY) has been successfully undertaken on intact breast tissues and FNA. Representative COSY spectra of lesions of malignant, ductal carcinoma *in situ* and benign pathologies are shown in Figure 3A, 3B and 3C, respectively. The COSY spectrum of an FNA from a malignant lesion is shown in Figure 4. The majority of crosspeaks assigned in adenocarcinoma from other organs have been identified in the spectra of adenocarcinoma of the breast (Table 1) including those from lipid, cellular metabolites and cell surface fucose (Figure 5). Data collection has now commenced.

FNA of lymph nodes from breast cancer patients and control subjects

The FNA technique has been extended to include biopsies from the axillary lymph nodes of breast cancer patients. Representative 1D MR spectra of tissue and FNA specimens of axillary nodes are shown in Figure 6. The signal to noise and spectral resolution of the FNA spectrum is adequate to allow visual and multivariate analysis of the data. Data collection has now commenced.

New Collaborations on MRS /FNA breast study

A collaborative program has been established between the Karolinska Institute in Sweden and the IMRR.

The Karolinska Institute will carry out an independent study.

Surgical students now being trained on this program

The IMRR is now training surgeons on this project under the Australian NH&MRC Clinical Centres of Excellence award.

SUMMARY OF PROGRESS from April 29, 1998 to April 28, 1999

Publications:

1. Mountford CE, Breast Cancer and MRS, *Life Sciences*, 10 (5), 22, 1998.
2. Malycha PL and Mountford CE, Magnetic Resonance Spectroscopy and Breast Cancer. *Australian and New Zealand Journal of Surgery*, 68, 859, 1998.
3. Himmelreich U, Mackinnon WB, Barry P, Russell P, Neilson G and Mountford CE. Proton MR for Pathological Cancer Diagnosis in Fine Needle Biopsy Specimen of Breast Cancer Lesions. *Proc. Int. Soc. Magn. Res.*, 788-789, 1998.
4. Lo W, Dowd S, Malycha P, Russell P, Mountford CE and Lean CL, Detection of Metastases in Lymph Nodes of Breast Cancer Patients Using Proton Magic Angle Spinning (MAS) MRS. *Proc. Int. Soc. Mag. Res. Med.*, 1: 451, 1999.

Experimental Progress

Increase in Number of Breast FNA Biopsies

A further 129 FNA biopsies were collected by participating surgeons during 1998/1999. Sixty of these specimens were deemed unsuitable for analysis due to having thawed during transport to the MRS facility (a result of incorrect handling by couriers). Of the 69 remaining FNAs, 45 were analysed using the standard one Dimensional MRS protocol (Figure 7A) and 24 were analysed using a newly developed automated MRS protocol (Figure 7B) in preparation for transfer of this technology to the clinical setting. The automation protocol is outlined in Figure 8.

Ductal Carcinoma In Situ Specimens.

MRS FNA data from an additional 6 ductal carcinoma *in situ* specimens were obtained and correlated with the step serial section histopathological data from the tissue surrounding the FNA site. By MRS criteria, 3 of these DCIS specimens were benign and 3 were malignant (Figure 7A). No standard histological features, including comedonecrosis or microinvasion, confirmed the MR diagnosis. However, of the three carcinoma *in situ* specimens ranked as malignant, two were obtained from re excisions from women with previous breast cancers. One of these women had nodal metastases present at the time of re excision. Confirmation of the MR diagnosis requires follow up of these patients and correlation with clinical outcomes.

Multivariate Analysis of One Dimensional MR Data

Analysis of FNA MR spectra by visual inspection distinguishes between malignant and benign pathologies with a high sensitivity and specificity only when the signal to noise ratio (SNR) of the spectra exceeds 25 (Figure 9).

Computerised consensus diagnosis (CCD), overcomes this limitation, successfully distinguishing malignant and benign pathologies, irrespective of SNR (Table 2). Computerised consensus diagnosis also predicts the presence or absence of (A) vascular invasion and (B) lymph node involvement with high degrees of sensitivity and specificity (Table 2).

FNA specimens analysed by MRS were deemed unsuitable for multivariate analysis if the spectra displayed incomplete suppression of the resonance from residual water or the presence of contaminant peaks. The problem of inadequate water suppression has now been overcome with the refinement and successful application of a new MR pulse sequence (Figure 10).

Two Dimensional (2D) MR Assessment of Breast Tissue and FNA Biopsies

Two dimensional (2D) COSY MRS was unable to discriminate between breast tissues of varying pathologies due to large contributions from lipid in most spectra which prevent metabolites present in lower concentrations being recorded (Table 3). 2D COSY (NS=48, NE=200, 4h experimental time) also fails to discriminate FNA specimens of varying pathologies due to inadequate signal to noise in the majority of spectra (Table 4). We are now investigating whether by increasing the number of accumulations acquired in each experiment (NS=192, 12h experiment time) the problem of inadequate signal to noise in FNA spectra can be overcome. A new MR technique, magic angle spinning (MAS), is also under investigation as a means of obtaining increased chemical information from intact breast tissue.

Assessment of Lymph Nodes from Breast Cancer patients and control subjects

MRS on FNA (n=15) discriminates between malignant and reactive nodes based on the ratio of intensities of resonances at 0.89ppm and 0.90ppm (resolved in spectra following resolution enhancement using a Gaussian-Lorentzian transformation, GB=0.08, LB=-7.0) plotted against the ratio of intensities of resonances at 3.2ppm and 0.9ppm (Figure 11). The discriminant capacity of MRS for detecting metastatic disease in human lymph nodes is supported by the MAS MRS data from intact lymph node tissues (n=17) shown in Figures 12 and 13. Malignant nodes were distinguished from reactive nodes based on the peak height ratio of the smallest to largest peaks resolved in the 0.9 ppm resonance multiplied by the peak height ratio of the choline and creatine resonances at 3.2 and 3.0ppm, respectively (Figure 13). Three of four axillary nodes, from breast cancer patients, which were clinically suspicious but diagnosed cancer free on routine histopathology had an MAS MRS ratio consistent with reactive nodes. The fourth node in this category was by MAS MRS criteria, malignant.

Surgeons being trained on this program

Dr Laurence Gluch, a fully trained surgeon, has in 1999 commenced a PhD program on this project supported by the Australian NH&MRC Clinical Centres of Excellence award.

SUMMARY OF PROGRESS from April 29, 1999 to April 28, 2000

Publications:

1. Mountford CE. Spectroscopy Rediscovered: The Gold Standard for the Next Millennium, Plenary Session, *Proc. Int. Soc. Magn. Reson Med.* #290, 1999.
2. Gluch L, Lean CL, Russell P, Malycha P, Mountford CE, Somorjai RL, Dolenko B, Nikulin A, Smith ICP. Pathology, Vascularisation and Nodal Involvement Determined using Magnetic Resonance Spectroscopy from a Single Aspiration Breast Biopsy. *Proc. Int. Soc. Magn. Reson Med.*, #1946, 2000.
3. Lean CL, Russell P and Mountford CE. Magnetic Resonance Spectroscopy: A New Gold Standard for Medical Diagnosis. *Journal of Women's Imaging*, 2 (1): 19-30, 2000.
4. Mountford CE, Somorjai RL, Gluch L, Malycha P, Lean CL, Russell P, Bilous M, Barraclough B, Gillett D, Himmelreich U, Dolenka B, Nikulin A and Smith ICP. Magnetic Resonance Spectroscopy on Breast Biopsy determines Pathology, Vascularisation and Nodal Involvement. Submitted. (See Appendix VII).

Experimental Progress

NB: Multivariate mathematical analysis methods employed, including computerised consensus diagnosis (CCD), will henceforth be called statistical computerised strategies (SCS).

Increase in Number of Breast FNA Biopsies

A further 127 FNA biopsies were collected by participating surgeons during 1999/2000. Eighty four of these specimens were collected by a single surgeon (Dr Peter Malycha) as paired samples from 42 patients, the first FNA taken through the skin immediately prior to surgery and the second FNA taken from the excised lesion as previously described (See Methods). One similar pair of FNA were collected by a separate surgeon (Dr Margaret Pooley) but were excluded from SCS analysis so that data from a single aspirating surgeon could be assessed. Nine single FNA specimens were also obtained from surgeon Dr Peter Malycha. The remaining 32 FNA specimens were obtained by a third surgeon (Prof David Gillett) from individual patients attending a breast clinic. These patients were not sedated at the time of FNA. Thus, problems associated with taking the FNA in a clinical setting, including alcohol contamination and reduced FNA cellularity were addressed. One hundred and twelve of 127 FNA, including all paired data sets undergoing SCS, were analysed using the new 1D MR pulse sequence described in the 1998/99 Progress Report (Figure 10) to ensure adequate water suppression. The remaining 15 FNA were analysed using the standard one dimensional (1D) MRS protocol.

Ductal Carcinoma *In Situ* Specimens.

MRS FNA data from an additional 2 ductal carcinoma *in situ* specimens were obtained and correlated with the step serial section histopathological data from the tissue surrounding the FNA site. By MRS criteria, one DCIS was malignant and the other benign. No standard histological features, including comedonecrosis or microinvasion were found for either specimen. Confirmation of the MR diagnosis requires follow up of these patients and correlation with clinical outcomes.

Multivariate Analysis of One Dimensional MR Data

Proton MRS combined with SCS can: A) distinguish malignant and benign pathologies with a sensitivity and specificity of 92% and an accuracy of 93%; B) determine the presence or absence of vascular invasion with a sensitivity and specificity of 84% and 100%, respectively and an accuracy of 92%; C) predict lymph node involvement with a sensitivity and specificity of 96% and 94%, respectively and an accuracy of 95%, all from a single FNA from the primary breast lesion. This work is described in a manuscript recently submitted for publication (Appendix VII).

The use of SCS is not possible for MR data displaying incomplete suppression of the resonance from residual water or the presence of contaminant peaks. We have now confirmed from a new data base of 112 FNA that inadequate water suppression in the MR spectra can be overcome using the new MR pulse sequence detailed in the 1998/99 Progress Report (Figure 10).

Proton MR spectra from 64 FNA from 32 patients (32 pairs of FNA obtained A. through skin immediately prior to surgery and B. through the excised lesion at the time of surgery from the same patient) are now undergoing SCS analysis. These data will determine whether or not the method of collection effects the MR data.

International Workshop on MRS of the Breast Hosted by the IMRR

Proton MRS combined with SCS can, from analysis of a single FNA from a primary breast lesion: A) distinguish malignant and benign pathologies with high sensitivity and specificity, B) determine the

presence or absence of vascular invasion and c) predict lymph node involvement (Appendix VII). In order to facilitate the rapid inclusion of this technology into breast clinics world wide the IMRR hosted a Workshop on MRS of the Breast in March 2000 (Program attached - Appendix VIII). Attendees included IMRR staff and local collaborators and representatives from three of the four participating international sites: the Karolinska Institute, Stockholm, Sweden; Beth Israel and Deaconess Hospital, Boston USA; Royal Adelaide Hospital, Adelaide, Australia. A protocol was developed at this meeting for a multi-centre trial to verify the MRS method specific to breast disease (Appendix IX). This trial is now commencing world wide.

Two Dimensional (2D) MR Assessment of Breast Tissue and FNA Biopsies

Two dimensional (2D) COSY spectra (NS=192, 12hr experiment time) were collected for 30 breast FNA. Despite the increased number of accumulations the signal to noise of these spectra was not adequate to provide useful data in addition to that obtained using 1D MRS.

Two dimensional COSY "magic angle spinning" MRS was used to assess 15 breast tissue samples. However, here again the chemical information obtained in the presence of large amounts of lipid was not sufficient to warrant use of this technique in addition to 1D MRS to discriminate between breast tissues of varying pathologies.

Assessment of Sentinel Lymph Nodes from Breast Cancer Patients

The MRS on FNA method used to discriminate between malignant and reactive nodes reported in the Annual Progress Report for 1998/99 is now being developed for the assessment of sentinel lymph nodes from breast cancer patients. Ninety two FNA from sentinel nodes were collected during April 1999- April 2000.

Surgeons Being Trained on this Program

Dr Laurence Gluch, a fully trained surgeon, has now commenced the second year of his PhD program on this project supported by the Australian NH&MRC Clinical Centres of Excellence award. Mr Gluch attended the annual meetings of the International Society for Magnetic Resonance in Medicine in Denver, USA and the Australasian Society of Breast Disease, Gold Coast, Australia where he presented results from this program. He also attended the International Workshop on MRS of the Breast outlined above.

BODY OF PROPOSAL

PURPOSE

1. To assess the sensitivity and specificity of ^1H MRS (*ex vivo*) in the detection of neoplasia in breast, based on altered cellular chemistry.
2. Correlate alterations to MRS detectable cellular chemistry associated with breast tumour development and progression with established clinicopathological criteria.
3. Ascertain MRS markers which correlate with known clinical, epidemiological and genetic risk factors to be used to identify women at-risk of developing breast cancer.

HYPOTHESES

Proton magnetic resonance spectroscopy can:-

1. Identify altered cellular chemistry in breast tissue independent of method of biopsy eg. open biopsy or fine needle biopsy.
2. Distinguish invasive cancer from normal breast tissue.
3. Distinguish between breast cancers of different type (e.g. lobular or ductal) and grade.
4. Distinguish between progressive and non progressive 'carcinoma *in situ*'.
5. Distinguish between 'normal' breast tissue and 'normal' breast tissue from women with differing risk factors for breast cancer (including age, pre- and post menopausal status, cyclic hormonal effects, genetic predisposition and family history).
6. Identify MRS markers of potential for malignancy in morphologically normal tissue from high risk patients.
7. Independent of histopathology, predict tumour behaviour and clinical outcome (e.g. response to therapy, patterns of metastases) and thereby be a significant predictor of patient survival.

EXPECTED RESULTS

- Expertise in optimal handling and methodological protocols for assessment of breast tissue.
- An MRS data bank on a full spectrum of breast tissue from normal (ie. benign) to high grade malignancy. This will allow discrimination of new subsets of cancer-bearing patients for streaming into different management regimes with attendant improved psychosocial, cost efficiency and clinical outcomes.
- Identification of new cellular chemistry parameters of tumour development in the breast.
- Definition of MRS markers which correlate with a predisposition for breast cancer development.
- Statistical verification of MR diagnostic criteria.

RESEARCH PLAN AND PROGRESS TO-DATE

(Results and progress during the last year are in bold)

NB: Multivariate mathematical analysis methods employed, including computerised consensus diagnosis (CCD), will henceforth be called statistical computerised strategies (SCS).

A. SAMPLE PREPARATION

Objective 1.

Optimise specimen handling and MR data acquisition protocols.

Rationale: Breast tissue contains substantial levels of fat which mask the diagnostic markers of tumour development and progression. Removal of as much of this fat component as possible from the biopsy prior to MRS analysis is both achievable and necessary.

Results:

1996-1997

MRS handling criteria are established and global specimen handling protocols determined to ensure adequate histological correlation of MRS data on the test samples (see appendix II).

1997-1998

Effect of Time Stored at -70°C on FNA MRS Correlation with Pathology: To date 13 FNA were stored at -70°C for greater than six months but less than 22 months and then assessed by MRS according to the standard protocol outlined in Appendix II. The 3.25/3.05 ppm MR intensity ratio was correlated with the pathology of the lesions (Figure 1B) and the results compared with those obtained for 15 FNA assessed after storage for greater than 6 weeks but less than six months (Figure 1A). The MRS correlation with pathology was maintained for FNA under six months old. Samples stored for greater than six months were found to be not suitable for MRS assessment due to a higher than expected number of false negatives. This albeit small study indicates that both benign and malignant specimens biodegrade during longer storage and the resonance intensities of the diagnostic marks are no longer reliable.

Objective 2.

Ascertain if the same MR criteria can be obtained on fine needle biopsy or aspirate.

Rationale: If the same MR information can be obtained from a fine needle aspirate or needle core biopsy, open surgery may not be a prerequisite for determining the diagnostic parameters.

Results:

1996-1997

Breast: A previous study of MRS analysis on FNB of thyroid had established that as few as 10^6 cells were required to obtain one-dimensional MR spectroscopic data with adequate signal-to-noise in less than 15 minutes (256 accumulations) (39). To achieve an adequate breast sample, FNB was performed using multiple (typically 6) aspirated passes (23 gauge needle) either through the resected lesion *ex vivo* (n=129) or *in vivo* (n=89) after lesion identification during open biopsy. These techniques could be guaranteed to provide sufficient cells from the lesion and in addition, allowed the aspiration site to be identified at excision. Tissue from the aspiration site (3mm^3) was collected for correlative histopathology. Prior to the ^1H MRS experiment, each FNB specimen was thawed and transferred directly to a 5 mm MRS tube. The

volume was adjusted to 300 μ l with PBS/D₂O where necessary. The sample tube was fitted with a capillary insert containing 60 μ l para-aminobenzoic acid (10 mM in PBS/D₂O) as an external standard.

1997-1998

Effect of FNA Collection Method (*in vivo* versus *ex vivo*): Specimen collection protocols have been established for obtaining FNA biopsies *in vivo* (prior to surgical excision of the lesion). This is an important step towards clinical trial of the MRS technology. A representative proton 1D MR spectrum of an FNA biopsy collected from a breast lesion *in vivo* is shown in Figure 2. The signal to noise and resolution of the *in vivo* spectra is of sufficient quality to allow correlation with histopathology on the basis of criteria generated from both visual inspection and multivariate analysis. This method of specimen collection is now used in the 1998-1999 year where clinically appropriate.

Lymph Nodes: Attempts to detect malignant cells in lymph nodes using 1D MRS have been limited by the high fat content of the tissue and the low spectral resolution. Using 2D COSY spectroscopy lymph nodes containing cancer cells were detected based on changes in the intensities of crosspeak resonances from lactate, choline, fucose and amino acids and these MR results were confirmed by xenographing the nodes into nude mice (41). COSY experiments are, however, time consuming (4-5 hours). Alternatively, the contribution from fat to the 1D MR spectrum can be reduced, as for breast tissues (Objective 1), by assessment of fine needle aspiration (FNA) biopsies of the lymph node tissue.

The proton MR spectrum obtained from solid lymph node tissue (Fig 6A) is of poor spectral resolution with predominant resonances from lipid at 0.89, 1.3, 1.7, 2.0, 2.2 and 5.37 ppm. These peaks represent $-\text{CH}_3$, $(-\text{CH}_2-)_n$, $-\text{CH}_2-\text{CH}_2-(\text{CH}_2)_n$, $(-\text{CH}_2)_n-\text{CH}_2-\text{CH}=\text{}$, $-\text{COO}-\text{CH}_2-$ and $-\text{CH}=\text{CH}-$, respectively. Figure 6B shows the ^1H MR spectrum of the FNA biopsy. A marked improvement in the spectral resolution of the lipid resonances at 0.89, 1.3, 1.7, 2.0, 2.2 and 5.37 ppm is achieved which allows a number of other resonances of potential diagnostic importance to be observed. In particular the resonance that represent $-\text{CH}_2-\text{CH}_2-(\text{CH}_2)_n$ at 1.7 ppm is a distinct peak which was only observed as a shoulder in Fig. 6A. The increase in spectral resolution is further evident in the choline peak at 3.2 ppm. In Fig. 6A this peak is buried in noise while in Fig. 6B the same resonance is resolved into its component (see insert Fig. 6B). The component peaks at 3.20, 3.21 and 3.27ppm, representing choline, phosphocholine and inositol respectively, can all be distinguished.

1998-1999

MRS FNA of Lymph Nodes: FNA specimens from 20 malignant and 25 reactive lymph nodes have been analysed using 1D ^1H MRS. Of these, only 33% of FNA specimens (3 malignant and 12 reactive) gave rise to MR spectra with adequate signal to noise ratios. The initial high rate of inadequate FNB specimens is attributed to technical difficulties encountered by participating surgeons in acquiring sufficient cellular material from smaller nodes in the initial work up phase of this project.

Analysis of the 15 spectra with adequate signal to noise ratios show MRS on FNA discriminates between malignant and reactive nodes based on the ratio of intensities of resonances at 0.89ppm and 0.90ppm (resolved in spectra following resolution enhancement using a Gaussian-Lorentzian transformation, GB=0.08, LB=-7.0) plotted against the ratio of intensities of resonances at 3.2ppm and 0.9ppm (Figure 11).

Magic Angle Spinning (MAS) MRS of Lymph Nodes: The discriminant capacity of MRS to detect metastatic disease in human lymph nodes is supported by MAS MRS data from intact lymph node tissue (n=17) shown in Figures 12 and 13. The use of MAS significantly improves the resolution of proton 1D MR spectra of human axillary nodes, reducing the line width at half height of the 1.3ppm resonance from

30Hz (data not shown) to 8Hz (Figure 12B) and resolving this resonance and that at 0.9ppm into separate components (Figure 12B, insert). Resonances, assigned from 2D COSY, include lipid and amino acids (0.9ppm), lipid and lactate (1.3ppm), creatine/phosphocreatine/lysine (3.0ppm) and choline metabolites (3.2ppm). Malignant nodes were distinguished from reactive nodes based on the peak height ratio of the smallest to largest peaks resolved in the 0.9 ppm resonance multiplied by the peak height ratio of the choline and creatine resonances at 3.2 and 3.0ppm, respectively (Figure 13). Three of four axillary nodes, from breast cancer patients, which were clinically suspicious but diagnosed cancer free on routine histopathology had an MAS MRS ratio consistent with reactive nodes. The fourth node in this category was by MAS MRS criteria, malignant.

Axillary lymph node involvement is a key prognostic indicator for breast cancer, yet accurate assessment of nodes is limited by sampling and observer errors. Here we demonstrate that MAS MRS has the potential to provide fast and accurate assessment of human axillary nodes from breast cancer patients and thus can significantly improve the diagnosis and management of this disease.

1999-2000

A further 127 FNA biopsies were collected by participating surgeons during 1999/2000.

Effect of FNA Collection Method (in vivo versus ex vivo): Eighty four FNA biopsy specimens were collected by a single surgeon (Dr Peter Malycha) as paired samples from 42 patients, the first FNA taken through the skin immediately prior to surgery and the second FNA taken from the excised lesion as previously described (see Methods). One similar pair of FNA were collected by a separate surgeon (Dr Margaret Pooley) but were excluded from SCS analysis so that data from a single aspirating surgeon could be assessed. Nine single FNA specimens were also obtained from surgeon Dr Peter Malycha.

For 6 of the 42 pairs of FNA one or both of the MR spectra were unusable for SCS analysis due to a poor signal to noise ratio or inadequate shimming of the magnetic field. A further 4 pairs were unable to be analysed due to equipment breakdown.

Proton MR spectra from the remaining 64 FNA (32 pairs) from 32 patients are now being analysed using SCS. These data will determine whether or not the method of collection effects the MR data.

Through Skin FNA Collection in a Clinical Setting: Thirty two FNA specimens were obtained by surgeon, Prof David Gillett, from individual patients attending a breast clinic. These patients were not sedated at the time of FNA. Thus, problems associated with taking the FNA in a clinical setting were addressed. The first 9 of the 32 FNA were contaminated with ethanol used to prepare the skin prior to FNA. This problem was overcome by waiting for 1-2 minutes after preparing the skin before commencing FNA. Six of 32 FNA were inadequate due to reduced cellularity. This was ascertained to be the result of patient discomfort due to multiple passes being performed. Communication and reassurance by the surgeon were found to be key elements in overcoming this problem. The signal to noise and resolution of the remaining 17 FNA spectra were of sufficient quality to allow correlation with histopathology on the basis of criteria generated from both visual inspection and SCS analysis.

Assessment of Sentinel Lymph Nodes from Breast Cancer Patients: The MRS on FNA method used to discriminate between malignant and reactive nodes reported in the Annual Progress Report for 1998/99 is now being developed for the assessment of sentinel lymph nodes from breast cancer patients. Ninety two FNA from sentinel nodes were collected during April 1999-April 2000. The first 34 samples were not useable due to incorrect collection protocols used by new surgeons on the program. The remaining 58 FNA were analysed using the standard 1D MRS protocol. Signal to noise and spectral resolution were adequate for SCS analysis. However, a distortion of resonances in the spectral region 0.5 - 1.0 ppm was observed in some spectra. Resonances from the blue dye present in the sentinel nodes was confirmed not to be the cause of the distortion and not to interfere with other MR resonances of potential diagnostic importance. We are now investigating the effect of the radioactive tracer on the MR spectrum.

B. CORRELATION WITH HISTOLOGICAL CRITERIA

Objective 3.

Identify MRS criteria to facilitate distinction between resected carcinoma and normal tissue.

Rationale: The primary data base will be obtained from this section of the study. The 1D and 2D MR data are likely to contain the majority of resonances that need to be assigned to chemical species, biological criteria and clinicopathological criteria.

Results:

1996-1997

All pathological and MRS analyses were undertaken in a blinded study. Correlation of MRS data with clinicopathological criteria were made after all reports were filed. 218 FNB and tissue specimens for MRS analysis and correlative histopathology respectively were obtained from 191 consecutive patients undergoing diagnostic biopsy or definitive treatment (lumpectomy, quadrantectomy or mastectomy) for histologically proven invasive breast cancer. Indications for surgery included mammographically detected impalpable lesions as well as palpable mass lesions which were suspicious by mammography, FNA cytology and/or clinical examination. The age range of patients was 20 to 81 years (mean \pm SD, 52 \pm 14). Where mastectomy for invasive carcinoma was performed, control specimens of macroscopically uninvolved breast tissue (which was later confirmed histologically) were obtained in the same patient (n=27). The MR experimental methods, data processing and analysis, peak assignment procedures and histopathological assessment protocols are described in detail in Appendix II.

Invasive carcinoma was identified by resonances at 3.25 ppm attributable to choline-containing metabolites (Appendix II, Figure 2). A discrimination between invasive carcinoma (n=82) and normal or benign tissue (n=106) was made based on the intensity of the 3.25 ppm resonance standardised to the resonance intensity at 3.05 ppm containing contributions from creatine, phosphocreatine and lysine ($p < 0.0001$, Mann-Whitney U test)(Appendix II, Figure 3). A receiver operating characteristic (ROC) curve using this intensity ratio is shown in Appendix I, Figure 4.

Of 106 benign samples, 102 gave a 3.25/3.05 ppm intensity ratio of less than 1.7. Four false positive results were obtained from 3 palpable fibroadenomas, none of which had FNA cytology performed, and 1 lesion comprising moderate ductal hyperplasia. The diagnoses were based on correlative histopathology. FNB from 4 of 82 carcinoma had a ratio of < 1.7 . Correlative histopathology from the aspiration site showed that one sample had only benign fibrocystic changes in this region. The other three samples were confirmed as invasive carcinoma but with a marked inflammatory cell infiltrate.

Clinical Correlations: All cases presenting as mammographically suspicious (n=56), mammographically negative (n=23), or non-diagnostic (n=14) or atypical/suspicious (n=25) FNA cytology, were accurately categorised by MRS (as benign or malignant) as confirmed by histopathology

on tissue excised from the aspiration site. MRS on F.B. correlated 96% with the final histological diagnosis of benign lesions (Appendix II, Table 2) and yet, biopsy was performed because of clinical (34%) and/or mammographic features (45%) and/or FNA cytology (31%). MRS on F.B. correlated with a malignant histological diagnosis in 95% of cases (Appendix II, Table 2). While combined triple assessment indicated biopsy for all the malignant cases studied, no single pre-operative modality was an improvement on MRS in identifying malignancy (physical examination 84%, mammography 82%, FNA cytology 92%).

1998-1999

A further 129 FNA were collected from participating surgeons during 1998/99 for MRS analysis. Of these, 60 were unsuitable for inclusion in the study due to incorrect handling by couriers (allowed to thaw) during transport to the MRS facility. Of the 69 remaining FNAs, 45 were examined using the standard 1 Dimensional MRS protocol (Figure 7A) and 24 were analysed using a newly developed automated MRS protocol (Figure 7B) in preparation for transfer of this technology to the clinical setting.

Automation of MRS Protocol: Proton MRS *ex vivo* has the potential to be a very useful diagnostic tool available for routine use in the clinic. There has to date, however, been no application of ¹H-MR spectroscopy in routine clinical pathology due to the technical complexity of acquiring and processing the proton MR spectra.

As a first step in overcoming this problem we have now developed an algorithm to enable high resolution proton MR spectra of breast FNA to be obtained using fully automated acquisition and processing procedures. The main components of the automation algorithm are shown in Figure 8.

Twenty four FNA specimens were analyzed using this algorithm. The only tasks performed by the operator were placement of the MR tube in the magnet and typing the start command. No manual probe tuning was necessary during the 40 days over which the data was collected.

The MRS data were correlated with the post-operative hospital pathology for each patient. Malignant and normal or benign tissue were discriminated with a sensitivity and specificity of 100% and 78%, respectively, using the standard protocol, ie. the ratio of MR resonances from choline-containing metabolites (3.25 ppm) and creatine (3.05 ppm). A MRS ratio < 1.7 was recorded for all normal/ benign lesions. Only three samples yielded spectra that could not be used due to a low signal-to-noise ratio.

These results show that the MRS FNA method may be readily developed into an easy-to-use diagnostic tool for routine clinical use. Further developments required include a fully automated determination of diagnostic signals using computerized consensus diagnosis and provision of this diagnosis.

Objective 4.

Establish whether MRS can identify differences between variants of breast cancer (e.g. lobular and ductal carcinoma) which reflect altered biological behaviour (e.g. likelihood of response to treatment or location of secondary tumours).

Rationale: Lobular and ductal carcinoma have quite different patient outcome. Ductal carcinoma will usually metastasise to the liver, brain, bone, lungs etc whereas lobular carcinoma frequently metastasises to unusual sites. If spectral differences exist between these two types of breast carcinoma and can be assigned to specific biological criteria eg. altered cell surface glycosylation which could reflect altered immunosuppression (50), ability to lodge at different sites (51-53), or adhesion properties (54), the patient outcome could be rationalised at an early stage in the diagnosis and treatment altered accordingly. The

reasons for variable survival of women with established metastatic disease may be manifest in the cellular chemistry of the carcinoma cells.

Experimental: Underway. This aspect of the project has been delayed due to a shortage of lobular specimens. Insufficient numbers of lobular cancers have been obtained to undertake SCS analysis. Specimen collection will continue.

Objective 5.

Correlate MRS properties with the established pathological characteristics of breast epithelial hyperplasia and neoplasia.

Rationale: It is important to identify which MRS characteristics are consistent with established pathological criteria. MRS will be used, as an adjunct to current histopathology, with each method contributing to a more thorough and refined diagnosis and patient management.

Experimental: Underway. Insufficient numbers of hyperplasia specimens have been obtained to undertake SCS analysis. Specimen collection will continue.

C. CAN MRS REDEFINE THE PRECURSOR STATES OF BREAST NEOPLASIA?

Objective 6.

Compare simple hyperplasias, atypical hyperplasias and malignant tumours. Does MRS identify sub groups or categorise these histological states differently?

Rationale: The strength of the MR method as demonstrated in its application to other organs, was the identification of alterations to cellular chemistry which were not morphologically manifest, or which discriminated between subsets with overlapping or identical histological appearances (e.g. follicular adenoma and carcinoma of thyroid).

Experimental: Underway. Insufficient numbers of hyperplasia specimens have been obtained to undertake SCS analysis. Specimen collection will continue.

Objective 7.

Assess resected tissue containing morphological carcinoma *in situ* to determine if the MR method can separate those, if any, which contain cells committed to invasion from those that cannot progress at that time.

Rationale: Diagnosis of carcinoma *in situ* (CIS) of the human cervix relies on the pathologist confirming that the cells (which are morphologically indistinguishable from invasive cancer) have not invaded. MRS has clearly shown that CIS of the cervix does not have the same cellular chemistry as those cells which are invasive. Does CIS of the breast have a different cellular chemistry from invasive cells? Can the distinction be made between a CIS which has cellular capability to invade from those which have yet to develop fully invasive properties?

Results:

1996-1997:

Specimens reported as ductal carcinoma-*in-situ* (DCIS) by routine hospital histopathology were obtained and assessed by MRS. Samples containing only DCIS (10 high grade, 1 low grade) all gave MR ratios \leq

1.7 indicative of a low choline to creatine/lysine ratio similar to that obtained for benign lesions. Ductal cells had breached the basement membrane ($< 1\text{mm}$) in one or more foci of the entire DCIS specimen in the 4 samples denoted 'microinvasion'. This group as well as 2 samples of high grade DCIS with extensive comedonecrosis gave ratios > 1.7 similar to that obtained for malignant lesions.

1997-1998:

A further DCIS specimen was collected. By MRS criteria this specimen had a ratio greater than 1.7 making it malignant by MR criteria. Interestingly, the pathology indicated that the patient had invasive cancer elsewhere in the breast. This observation is consistent with data obtained on the prostate program where when cancer was present elsewhere in the organ a histologically benign specimen gave a MRS ratio consistent with malignant.

To further examine the extent to which MRS can record an adenoma-carcinoma sequence in breast neoplasia based on changes to cellular chemistry two dimensional (2D) correlated spectroscopy (COSY) techniques have been optimised for the assessment of intact breast tissue biopsies. Representative COSY spectra of lesions of malignant, ductal carcinoma *in situ* and benign pathologies are shown in Figures 3A, B and C, respectively.

There are in excess of 50 chemical species (crosspeaks) identifiable in the 2D COSY MR spectra of some invasive tumour biopsies and malignant cell lines (2). Many of these crosspeaks have been assigned (8). Those used in other organs as diagnostic markers include lipid, cell surface fucosylation and cellular metabolites.

Resonances from cell surface fucose are of particular interest in the characterisation of the malignant potential of preinvasive lesions. 2D COSY spectroscopy of adenomatous colorectal polyps displayed a progressive increase in the complexity in the spectral pattern from fucosylated species that correlated with the malignant potential of the adenoma, specifically with the histological degree of dysplasia.

Crosspeaks identified and assigned in the spectra of breast lesions including those from lipid, cellular metabolites and cell surface fucose (Figure 5) are summarised in Table 1.

1998-1999:

Two-dimensional MR analysis of breast FNA and tissue specimens has continued. Fifteen breast tissues have been analysed by two dimensional (2D) Correlated Spectroscopy (COSY). The resonances observed in the spectra of benign, malignant and DCIS (ductal carcinoma *in situ*) tissue specimens are summarised in Table 3. 2D COSY MRS fails to discriminate between breast tissues of varying pathologies due to large contributions from lipid in most spectra which prevent metabolites present in lower concentrations to be recorded. Sixteen breast FNA specimens have also been analysed using 2D COSY (NS=48, NE=200, 4h experimental time). Using these acquisition parameters, COSY also fails to discriminate FNA specimens of varying pathologies due to inadequate signal to noise in the majority of spectra (Table 4). We are now investigating whether by increasing the number of scans acquired in each experiment (NS=192, 12h experiment time) the problem of inadequate signal to noise in FNA spectra can be overcome. A new MR technique, magic angle spinning (MAS), is also under investigation as a means of obtaining increased chemical information from intact breast tissue.

A further six DCIS specimens have been examined by MRS during 1988/99 (Figure 7A). Three specimens had an MR ratio consistent with the presence of malignant disease. Examination of the tissue surrounding the FNA site for the six cases found no evidence of malignancy in any of the tissues. Review of the pathology of these patients found no standard histological features, including comedonecrosis or microinvasion, that confirmed the MR diagnosis. However, of the three carcinoma *in situ* specimens

ranked as malignant, two were obtained from re excisions from women with previous breast cancers. One of these women had nodal metastases present at the time of re excision. These data therefore suggests that MRS is reporting on changes in cellular chemistry that are not evident using histopathological techniques. Confirmation of the MR diagnosis requires follow up of these patients and correlation with clinical outcomes.

1999-2000:

MRS FNA data from 2 high grade ductal carcinoma *in situ* specimens were obtained and correlated with the step serial section histopathological data from the tissue surrounding the FNA site during 1999-2000. By MRS criteria, one specimen was malignant and the other benign. No standard histological features, including comedonecrosis or microinvasion were found for either specimen. Confirmation of the MR diagnosis requires follow up of these patients and correlation with clinical outcomes.

Two Dimensional (2D) MR Assessment of Breast Tissue and FNA Biopsies: Two dimensional (2D) COSY spectra (NS=192, 12hr experiment time) were collected for 30 breast FNA. Despite the increased number of accumulations the signal to noise of these spectra was not adequate to provide useful data in addition to that obtained using 1D MRS. Two dimensional COSY "magic angle spinning" MRS was used to assess 15 breast tissue samples. However, here again the chemical information obtained in the presence of large amounts of lipid was not sufficient to warrant use of this technique in addition to 1D MRS to discriminate between breast tissues of varying pathologies.

D. CAN MRS IDENTIFY CHEMICAL PROFILES ASSOCIATED WITH A PREDISPOSITION TO BREAST CANCER?

Objective 8.

Study morphologically normal tissue from women with differing risk factors for development of breast cancer.

Rationale: It is most likely that alterations to cellular chemistry are able to be documented in the above categories. Are there specific changes which can be identified in at-risk patients?

The effect of :-

- age,
- pre vs post menopausal status,
- cyclic hormonal effects,
- genetic predisposition,
- family history,

on the spectral profile will be considered in a retrospective study in association with the breast data bank registries.

Experimental: Insufficient numbers of normal tissue specimens have been obtained to undertake SCS analysis.

E. LONGITUDINAL STUDY

Objective 9.

Statistical and mathematical multivariate analysis to correlate MRS data with clinicopathological, epidemiological and genetic data.

Rationale: By examining tissue specimens by MRS, it may be possible to predict the precise progression of breast cancer in individual patients more accurately than histopathology.

Experimental: Underway

Results

1997-1998: MR spectra comprise a large number of variates, the intensities of absorption as a function of frequency. As many as 4000 variates may be available for each spectrum to be classified.

Extensive experience with classifying MR spectra indicated that better and more reliable classification is obtained if we only deal with the magnitude spectra. (The subjective spectral phasing step is completely eliminated.) Thus all analyses were conducted on the magnitude spectra.

Efficient application of multivariate analysis involves identification of the minimum number of variates required to classify the spectra accurately. We have developed a powerful and versatile preprocessing method (ORS_GA) that is Genetic Algorithm-driven and selects from the spectra those subregions that are optimally discriminatory (62). Reduction of the number of features to 1/5 – 1/10 of the number of samples per class is an essential requirement for creating reliable classifiers.

We used simple Linear Discriminant Analysis (LDA) with the selected optimal features (each such feature is the average intensity value of its spectral subregion). Classification robustness and reliability is achieved by our resampling procedure. This involves the random selection (with replacement) of ~half the samples from each class to use as the training set for the LDA, with the remainder serving as a test set. This is repeated N times (N is typically 500-1000), and the N sets of LDA coefficients averaged to produce a single, optimal set. (This combination of optimal region selection and resampled and averaged LDA has been successful for the accurate classification of MR and IR spectra of tissues and biofluids for a variety of diseases and disease stages.)

We applied the above classification strategy to the breast tissue spectra (water-suppressed, with 1250 data points per sample in the 0.5-4.0 ppm spectral range). There were 55 benign and 69 malignant samples. Three independent sets of 9 variates each gave very similar results: ~70% classification accuracy for the entire 124 spectra. However, when only the crisply (i.e., with probabilities to belong to a given class > 75%) classified spectra were considered, their classification accuracy reached ~90%. Unfortunately, the crisply classified spectra comprise only ~25% of the total number of spectra. Our consensus classifier, which uses the outputs of the three individual classifiers as inputs to another LDA classifier, increased the number of crisply classified spectra to 53%, but the overall accuracy of these remained at ~90%.

Further improvement requires:

- Increase in spectral data base to at least 100 per class.
- Removal of minor contaminants present in the storage D₂O solution present in some data files.
- Since the misclassified and fuzzily classified spectra are evenly distributed between the two classes, this suggests that better quality spectra would yield better classification results.
- Lipid suppression will also be tested.

1998-1999:

When the signal to noise ratio (SNR) of FNA spectra exceeds 25, analysis of spectral data by visual inspection distinguishes malignant and benign pathologies with a sensitivity and specificity of 100% and 80%, respectively (Figure 9B). However, if spectra with $SNR < 25$ are included in this analysis the sensitivity and specificity of the method falls to 83% and 72%, respectively (Figure 9A).

Multivariate analysis methods, specifically computerised consensus diagnosis (CCD), successfully distinguishes malignant and benign pathologies with high sensitivity and specificity for all FNA spectra including those with $SNR < 25$. The CCD method uses output probabilities from K independently created classifiers as input attributes to another classifier (e.g., Wolpert's combining rule). This produces classifiers whose output are much "crisper" than constituent classifiers. A high % crispness is of clinical relevance. For a 2-class problem, assignment that is considered "crisp probability" is that which equals or exceeds 75%.

Malignant or Benign FNAs (All SNR): Distinction of malignant (n=80) and benign (n=54) FNA for all data (including those with $SNR < 25$) using CCD required combination of two classifier outcomes, both of which had 8 subregions, but were normalised differently. A sensitivity and specificity of 92% was recorded with an overall accuracy of 93% and an overall %crisp of 96% (Table 2A). This was a significant improvement on the 83% sensitivity and 72% specificity calculated from these data analysed by visual inspection.

Malignant or Benign FNAs ($SNR > 25$): Applying the same protocol as above to FNA spectra with $SNR > 25$ further improves the sensitivity and specificity of distinguishing benign from malignant specimens to 98% and 94% respectively, with an overall accuracy of 96% and an overall %crisp of 99% (Table 2B).

Lymph Node Involvement and Vascular Invasion: The CCD method was further able to predict the presence or absence of A) lymph node involvement and B) vascular invasion from the MRS FNA data. Identifying lymph node involvement using CCD required the combination of two classifier outcomes, one of which had 4 subregions, the other 5 subregions. A sensitivity and specificity of 96% and 94%, respectively was recorded, with an overall accuracy of 95% and an overall %crisp of 95% (Table 2C). Identifying vascular invasion again required two classifier outcomes, each with 7 subregions. A sensitivity and specificity of 84% and 100%, respectively was recorded, with an overall accuracy of 92% and an overall %crisp of 94% (Table 2D).

Pulse Sequence Refinements To Improve Water Suppression: One severe problem with the observation of MR spectra of FNA in an aqueous suspension is the suppression of the residual water signal. Incomplete suppression of this signal was a major reason for FNA specimens being unsuitable for multivariate analysis. This problem has now been overcome with the refinement and successful application of a new MR pulse sequence.

Saturation of the water resonance by pre-irradiation is one of the most commonly and successfully used sequences to suppress the water signal. However, the remaining H_2O signal is in many cases still several times larger than any other signal in the MR spectra of FNA (Figure 10A) even with the use of 99% D_2O for suspension of cellular material. This not only yields a large unwanted water signal but also a distortion of the baseline in the spectra.

The remaining water signal and baseline distortion influence multivariate analysis outcomes and make this analysis in many cases impossible. A recent pulse sequence published by Hwang and Shaka (63) that delivers pure phase spectra with flat baselines, and that allows excitation into almost arbitrary profiles, can

overcome this problem. This pulse sequence is also useful for suppression of large solvent signals. The principle of the Hwang and Shaka pulse sequence is a simple echo sequence with a pulsed field gradient before and after the refocusing element. Any phase shift can be removed by applying this sequence twice. An introduction of a soft pulse into this $90E_x-G_1-90E_x-\tau-90E_x-G_1-G_2-90E_x-\tau-90E_x-G_2$ pulse sequence sharpens the excitation profile further.

We employed, similarly to Hwang and Shaka, a [soft $180E_x$ hard $180E_x$] refocusing element. This pulse sequence returns all magnetisation to its starting point except for the water signal (soft $180E_x$ pulse was applied for the water signal). The delay τ was set to either $500\mu s$ (Figure 10B) or $600\mu s$ (Figure 10C). The larger τ value yields a narrower excitation profile but causes a second excitation at a frequency $1/\tau$ from the water frequency. This yielded minor suppression of intensity for signals close to the water frequency for $\tau=500\mu s$ and minor suppression of intensity for signals on the edges of the spectral width ($>9\text{ppm}$ and $<1\text{ppm}$) for $\tau=600\mu s$, respectively. This loss in signal intensity is negligible compared to signal loss due to pre-saturation of the water resonance.

Water suppression in the FNA spectra using this new method was 15000 " 1000 compared to 600 " 100 with the pre-irradiation method. Another feature of this method is the presence of OH/NH protons that would normally be suppressed by a pre-saturation pulse applied to the water signal due to exchange of protons from the water with these from OH/ NH-groups. This can be seen in Figure 10 in the chemical shift range 6-9 ppm. These signals may provide additional information in a multivariate analysis.

1999-2000:

Proton MRS combined with SCS can: A) distinguish malignant and benign pathologies with a sensitivity and specificity of 92% and an accuracy of 93%; B) determine the presence or absence of vascular invasion with a sensitivity and specificity of 84% and 100%, respectively and an accuracy of 92%; C) predict lymph node involvement with a sensitivity and specificity of 96% and 94%, respectively and an accuracy of 95%, all from a single FNA from the primary breast lesion. This work is described in a manuscript recently submitted for publication (Appendix VII).

The use of SCS is not possible for MR data displaying incomplete suppression of the resonance from residual water or the presence of contaminant peaks. We have now confirmed from a new data base of 112 FNA that inadequate water suppression in the MR spectra can be overcome using the new MR pulse sequence detailed in the 1998/99 Progress Report (Figure 10).

International Workshop on MRS of the Breast Hosted by the IMRR: In order to facilitate the rapid inclusion of MR technology into breast clinics world wide the IMRR hosted a Workshop on MRS of the Breast in March 2000 (Program attached - Appendix XII). Attendees included IMRR staff and local collaborators and representatives from three of the four participating international sites: the Karolinska Institute, Stockholm, Sweden; Beth Israel and Deaconess Hospital, Boston USA; Royal Adelaide Hospital, Adelaide, Australia. A protocol was developed at this meeting for a multi-centre trial to verify the MRS method specific to breast disease (Appendix IX). This trial has now commenced world wide.

METHODS (*Methods additional to original submission are in bold*)

THE SELECTION OF PATIENTS

Biopsy specimens are to be obtained at the time of surgery on 500 patients undergoing surgery to excise benign and malignant lesions by participating surgeons. The indications for surgery include:

1. Mammographically detected impalpable lesions where malignancy cannot be excluded. Stereotactic fine needle aspiration biopsy will be the indicator for surgery.

In this subgroup approximately 50% will have small invasive cancers, 30% of patients can be expected to have benign lesions such as sclerosing adenosis whilst the remainder will have premalignant conditions like atypical hyperplasia through to carcinoma *in situ*.

2. Excisions of mass lesions which have been proven by mammography, fine needle biopsy, cytology and clinical examination to be malignant where breast conservation can be performed. This is generally for patients with smaller tumours but is dependent upon other factors such as breast size.

The attending surgeon would undertake to provide this follow-up through out this period with regular reports to a central registry office. Initially, patients will be recruited to the study consecutively. However, it is anticipated that, as the study progresses, selection criteria may be introduced to enhance the data base of specific subgroups of patients.

Lymph node biopsy specimens will be obtained at the time of surgery from the following patients undergoing surgery to excise benign and malignant lesions:

1. **All patients undergoing mastectomy or segmentectomy with axillary sampling or clearance.**
2. **Patients undergoing sentinel node sampling.**

THE PARTICIPATING SURGEONS

THE ROYAL ADELAIDE HOSPITAL

South Australian clinical material in the first instance will be obtained from the patients treated by one surgeon (PLM) from the Breast Endocrine and Surgical Oncology Unit at the Royal Adelaide Hospital. This unit treats 200 primary or new breast cancers per year and provides a dedicated service to a 900 bed teaching hospital on the campus of the University of Adelaide. All patients are treated according to protocol and management is reviewed by a multidisciplinary team prior to treatment. Patients are regularly entered into Australian, New Zealand and international trials where appropriate. The routine pathology service is provided by pathologists from the Institute of Medical and Veterinary Science who also provide a dedicated service and attend the multidisciplinary meetings.

The unit is undertaking research in the following fields:

1. Endocrine responses to oestrogen and progesterone in breast cancer with particular reference to insulin-like growth factor binding proteins.
2. The immunological response associated with human breast cancer with reference to tumour infiltrating lymphocytes (TILs) boosted by lymphocyte growth factors.
3. The evaluation of magnetic resonance imaging of the breast in two subgroups of patients:
 - a) young women with dense parenchymal breast tissue

- b) for the evaluation and accurate assessment of women with T3 breast cancers.
- 4. Dynamic doppler studies to evaluate vascular function in breast reconstruction using autologous tissues.
- 5. Angiogenesis in breast tumours determined by dynamic doppler.
- 6. A comparative study of carbon track localisation hook wire for mammographically detected impalpable breast lesions.
- 7. On-going evaluation of the following tumour markers: a) vimentin, b) cERB2, c) ER, d) PR, e) CA125, 153.
- 8. Open label study of high dose chemotherapy in patients with breast cancer using autologous peripheral blood, stem cells and G-CSF support.

All patients treated by the unit are reviewed at regular intervals according to protocols and information provided to the Cancer Registry.

The unit works in close association with the South Australian Breast X-ray Service which screens 30,00 women per year between the ages of 40 - 69 years. From 1994 the number of women screened will increase to 45,000 per annum. This service provides a constant supply of small tumours as 70% of lesions detected by the clinic are less than 2 cm in size. This service is closely audited and offers the service to the point of diagnosis with particular emphasis upon stereotactic fine needle aspiration biopsy and ultrasound guided needle biopsy. The program is independently audited.

This service can provide biological material ranging through benign, premalignant, ductal carcinoma *in situ* and carcinoma.

WESTMEAD HOSPITAL

The Breast Surgery Unit at Westmead Hospital is multidisciplinary involving all aspects of breast cancer patient care, with a major commitment to the management of patients with breast cancer irrespective of the stage of the disease at presentation. Approximately 150 patients with breast cancer are treated each year within the unit, with approximately 10% having advanced breast cancer and with an increasing number having *in situ* disease as the number of patients accessed through the Screening Unit increases.

The Unit is undertaking research in the following fields:

- 1. Since 1979 patients with operable breast cancer have had the option of a breast conservation approach involving 'lumpectomy', or 'quadrantectomy' with clear surgical margins, axillary clearance, and radiotherapy to the breast. A particular form of axillary clearance has been developed with improvements in the way the procedure can be taught to trainees and a video tape is in production and will be shown next year at surgical meetings of the Royal Australasian College of Surgeons.
- 2. Approximately 50 - 55% of the patients with operable breast cancer currently achieve breast conservation. An initial cohort of approximately 130 patients undergoing a breast preservation procedure have been followed and the results have been reported at 5 and 10 years. Patients needing mastectomy have been treated with total mastectomy and axillary clearance again using the axillary clearance technique developed by the unit.
- 3. A study has recently been completed to determine the value of the cERB2 antigen in predicting recurrence in node negative breast cancer patients followed for more than 5 years.
- 4. Apart from its own trials, the unit takes part in a number of international studies including the Zoladex trial for node positive breast cancer.
- 5. Ongoing evaluation of the following markers is being undertaken: a) ER, b) PR, c) ploidy, d) epidermal growth factor receptors.

The surgical team works closely with Radiation and Medical Oncologists, with the Breast Screening Unit and with the Cytology and Pathology Departments. Combined clinics are held and a major Breast Cancer Data Base has been established within the Radiation Oncology Department. Reports have been published on the efficacy of cytology prior to breast surgery, on the problems encountered with the procedures involved in breast conservation, and also the results from our 'Advanced Protocol Treatment' of advanced breast cancer at presentation. The senior surgeon is also the senior surgeon of the Screening Unit which now has considerable expertise in all aspects of Screening, and is recognised as a training unit for medical and paramedical professionals involved with Breast Screening. Surgical and radiotherapy trainees take part in the clinical program and for the past 12 months a 'Breast Fellow' has been appointed and this is a recognised post for the Royal Australasian College of Surgeons for postgraduate training breast surgery for young surgeons who have achieved Fellowship of the Royal Australasian College of Surgeons.

This service can provide biological material ranging through benign, premalignant, ductal carcinoma *in situ* and carcinoma.

CONCORD HOSPITAL

The Breast Endocrine Unit at Concord Hospital was established in 1986. It is composed of four dedicated surgeons working from a forty bed unit. It is closely associated with the University of Sydney and is involved in student teaching, registrar training and research.

Approximately 100 cases are seen per annum. The data collection is made on a standard protocol and processed using computer programs.

The unit is associated with the breast screening clinics of Central and Western Sydney. The principals attend these clinics and patients are then referred for treatment at Concord Hospital.

Multi-disciplinary meetings involving pathologists, radiologists and surgeons are held on a regular basis and a routine monthly review of all cases is undertaken. Standard protocols for breast treatment have been instituted and are followed by the treating surgeons.

Papers have been published on the accuracy of fine needle aspiration cytology in breast cancer patients, the incidence of mammography negative breast cancers seen through the clinic and the incidence of multi focal breast cancer.

This service can provide fewer specimens than the larger two centres but a strong collaboration between the MR Unit and surgeons at Concord Hospital is well established.

MAGNETIC RESONANCE SPECTROSCOPY

Magic Angle Spinning (MAS) MRS of Human Lymph Nodes: Sample Preparation: Axillary nodes were collected from breast cancer patients. Reactive jugular-digastric nodes were obtained from carotid endarterectomy patients. Nodes in 0.25 ml PBS/D₂O were frozen in liquid nitrogen and stored at -70°C for less than 3 months. For MRS, nodes washed in PBS/D₂O were placed in 4mm rotors (Bruker). ***¹H MAS MRS:*** A Bruker DRX 360 MHz spectrometer (Magnex magnet) and broad band MAS solid state probe were used with samples (37°C) spinning at 2.3 KHz. Water signals were suppressed by selective gated irradiation. 128 averages of 16K data points were acquired. Following MRS, nodes were fixed for histopathology. Data were zero filled to 32K and an LB=1Hz applied prior to Fourier transformation and polynomial baseline correction.

Sample Handling Tissues (tissue or FNA) obtained at surgery will be placed into a sterile tube containing pre-cooled (4 C) phosphate buffered saline in D₂O (PBS/D₂O) immediately after excision. Alternatively, biopsies will be snap frozen in liquid nitrogen, transported to the laboratory and stored at -70 C until ready for examination. Samples to be examined by MRS will be gently thawed, washed in PBS/D₂O (5 x 1 ml) and placed in a 5 mm MR tube containing sufficient PBS/D₂O to cover the biopsy. Placement within the transmitter/receiver coil will be ensured by either resting the biopsy on top of a plug of glass wool (5), or by suspending the biopsy inside an inner capillary tube of 2.5 mm diameter (10,34).

In the case of the capillary method, the external volume will be filled with 350 μ l of 1 mM p-aminobenzoic acid (PABA) in PBS/D₂O which serves as a chemical shift and concentration reference (34). **A third method is being included for the study of FNA from axillary lymph nodes. Samples suspended in PBS/D₂O (final volume 450 μ l) will be placed in specialised susceptibility matched 5 mm MR tubes containing a raised platform (Shigemi Co. NMR division).** As in the case of colorectal biopsies (21), prior to the MRS experiment the specimens will have excess adipose tissue and vasculature excised. Alternative methods such as passing the tissue through a metal sieve to trap the fat (see Page 17) will be explored.

F.B.: will be performed using 5 aspirated passes through the specimen with a 21 gauge needle (39). Previous studies (55) have shown that this combination gives the greatest number of cells, both single and in clumps, although it is not the preferred technique if clear cellular detail is required for accurate cytological examination. F.B. are washed with 2 x 1 ml PBS/D₂O. Between washes the samples are centrifuged (1000g for 5 min) and the supernatant discarded. The F.B. are suspended in PBS/D₂O (final volume 125 μ l) and then placed on top of a glass wool plug in a 5 mm MRS tube.

Data Collection Data will be collected on a Bruker AM360 wide-bore MR spectrometer equipped with an Aspect 3000 computer and a dedicated 5 mm or 8 mm ¹H probe. Temperature is maintained at 37 C using a Bruker VT1000 temperature regulation unit.

Pulse Sequences All pulse sequences to be used are functional in this laboratory at the time of application. Typical experiments are as follows:-

1D ¹H spectroscopy: 1D spectra (at 360 MHz) are obtained using a spectral width of 3600 Hz (10 ppm) and 8K data points. A relaxation delay of 2 sec is used during which gated decoupling (15 dB below 0.2 W) is applied for the final 1 sec to reduce the residual water signal (56). 128 transients are averaged.

CPMG: One-dimensional T₂- filtered experiments are performed using the Carr-Purcell-Meiboom-Gill pulse sequence (90_x-(-180_y-)_n-acquire) with an interpulse delay of = 1 msec and a 2 sec relaxation delay between acquisitions (48). Gated presaturation is used in the final 1 sec before data acquisition. 128 transients are collected using a spectral width of 10 ppm. T₂- filtered 1D experiments on biopsy specimens is performed using selected values of n. Typical values of n2 = 16, 480, 720, 960 ms.

2D ¹H-¹H COSY spectroscopy: Magnitude-mode COSY spectra are performed using a standard two pulse sequence with the two pulses separated by an incremented delay (57). The sweep width in the t₂ domain is 3000 Hz, and the size in the t₂ domain is 2K data points. The initial delay between the 2 pulses is 1 msec with an increment time of 334 sec. There is a relaxation delay of 1 sec before each accumulation during which the water resonance is presaturated using a CW irradiation power of 30 dB below 0.2 W. The number of time domain points collected in t₁ (free induction decays, FIDs) is subject to the viability limits of the sample. Tissue experiments consist of 180-220 FIDs, each of 32-48 transients (plus 2 dummy scans) over a total experiment time of three to five hours.

T₂-filtered CORrelation Spectroscopy: T₂-filtered correlation spectra are obtained by replacing the first pulse of a standard COSY pulse sequence with a CPMG sequence (48). Typical parameters are described above with n2 equal to 500 - 750 msec. The sequence removes crosspeaks arising from short T₂ species, like lipid, leaving only resonances from more mobile metabolites. The pulse sequence is useful in resonance assignment in specimens, like breast, which contain large quantities of lipid that can be reduced or removed using this technique.

Processing of MRS Data Data will be processed on a Bruker X32 (UNIX) Data Station or a Silicon Graphics Indigo 2 work station equipped with Bruker XWINNMR data processing software. The complexities of processing FIDs from biological material containing a wide range of T₂ relaxation

values (0.3 - 1.5 s) has been addressed in this laboratory (17). The processing parameters vary according to the MR visible chemicals of each tissue type.

1D spectroscopy: One dimensional spectra (1D and CPMG) are routinely processed using a line broadening of 3 Hz applied before Fourier transformation. Data are phase corrected using zero and first order phase correction and baselines are corrected using a fourth order polynomial baseline correction routine. Linewidths or ratios of peak heights are measured in 1D spectra.

2D spectroscopy: COSY data matrices undergo zero filling to 1K in t_1 , Fourier transformation and magnitude calculation ($(\text{Real}^2 + \text{Imaginary}^2)$) to give 1024 x 1024 real data points for each COSY spectrum. Sine-bell window functions are uniformly applied in the t_1 domain and Lorentzian-Gaussian window functions of varying width and maximum positions are applied in the t_2 domain prior to Fourier transformation as previously described (17). Crosspeak volumes and subsequent ratios to external or internal reference peaks will be measured as previously described (2,7,8,58).

Referencing peak height integrals, or peak heights, to those of an external standard with a constant concentration and T_2 relaxation value allows a semi quantitative concentration measurement. However, differences in crosspeak volumes can arise from either concentration changes or from changes in T_2 relaxation of the species. These two effects cannot be separated, but an increase in crosspeak volume will reflect either a chemical change or a reorganisation of the compound within the cell, and may still have diagnostic relevance.

MATHEMATICAL ANALYSIS OF MR DATA

NB: Multivariate mathematical analysis methods employed, including computerised consensus diagnosis (CCD), will henceforth be summarised as statistical computerised strategies (SCS).

Unprocessed MRS data will be transferred electronically via internet to the Silicon Graphics server at the Institute for Biodiagnostics, Winnipeg, Canada for a thorough mathematical analysis. The MR spectra will be prepared for analysis by means of the proprietary software ALLFIT (National Research Council of Canada), which provides appropriate baselines and integrals for each distinguishable peak. The spectra will then be preprocessed by formation of a correlation matrix to avoid problems due to widely disparate variate ranges. Principal component analysis will then be applied to the spectral regions of interest, in order to reduce the number of variables in the computation. One half the available data will be used as a training set, and the remaining half will be classified blindly. To make all analyses robust, the training set data will be analysed by the leave-one-out method *i.e.* train on $k-1$ of the k samples, classify the sample that was excluded from the training set, and repeat this k times, once for each sample.

Classification will be performed by a variety of methods, alone and in combination. The principal methods are linear and quadratic discriminant analysis (45), neural nets of various types (44), and genetic programming (46). Consensus analysis will be performed within each method using different regions of the spectrum and with combinations of methods, to increase accuracy of classification. Fuzzy logic methods will be applied where appropriate.

The steps in the multivariate analysis methods developed by Dr R Somorjai for the analysis of breast FNA specimens are as follows:

Preprocessing

- **Construct magnitude spectra**
- **Peak alignment of spectra**
- **Normalization to area of spectra**
- **Optimal region selection**

Classification

- Classifier is robust, reliable LDA
- Bootstrap-based cross-validation
- Classifier outputs are probabilities

Computerized Consensus Diagnosis (CCD)

- Uses output probabilities from independently created classifiers as input attributes to another classifier
- Produces crisper classifiers than constituent classifiers
- 2-class problem is considered crisp if probability greater than 75%

HISTOPATHOLOGY

Every MR sample will be assessed by one of two pathologists.

Expertise of the Pathologists The two pathologists participating in this study are Senior Hospital Consultants, each with over 25 years experience. Professor P. Russell is an acknowledged authority (59) and Dr J. Phillips is well recognised internationally in the field of aspiration cytology. Each pathologist has indicated that he/she is willing and able to make the necessary contribution in time to ensure the success of this project.

Rationale for the Extent of Histological Assessment

1. Primary correlation is obtained by comparing the MRS result with standard hospital histopathological diagnosis. This involves no additional time spent by the pathologist. Long-term storage of fixed tissue for later re-examination will be required.
2. The MRS sample is always examined **initially** by six "step-sections" 7 μ m sections (taken at 350 μ m intervals) at x 40 magnification. The intervening sections are mounted, stained and stored for future assessment if required. Tissue preservation, cellular content and presence of potentially confounding factors such as fat and inflammatory cells are reported in addition to the diagnosis. This involves an additional 5-6 minutes of pathologist time per specimen and is undertaken without reference to clinical or MRS data (in both MRS specimens and remainder of surgical specimen).
3. Where disagreement exists between MRS diagnosis and histological diagnosis, the MRS sample will be step-serially sectioned every 7 μ m (refer to 2) and examined by the pathologist in a blind study. To avoid bias other specimens will be included at the same time.

Tissue Preparation

Routine: Tissue is fixed in 10% buffered formalin or FAA (formalin:acetic acid:alcohol), paraffin embedded and sectioned, stained with haematoxylin and eosin according to standard protocols.

Serial sectioning: Routine sectioning of paraffin blocks will be at 7 μ m. Step serial sectioning of paraffin blocks will be undertaken on selected specimens.

Criteria for establishing firm histological diagnosis of cancer and its precursors in the tissues to be included in this study, will follow established guidelines. In general terms, the various pathological subtypes of cancer will be classified according to the relevant WHO International Histological Tumour Classification and, where difficult diagnostic problems are encountered (particularly for borderline malignancies), the guidelines in the following reference text will be used viz Page D.L. & Anderson T.J. (1990) Diagnostic Histopathology of the Breast. Churchill Livingstone, United Kingdom.

Quantification of Cell Types This will be performed usually on 'step' or 'full' serial sections and is only intended to provide an approximate guide to relative proportions of study tissues to background stroma and reactive inflammatory infiltrates etc.

STATISTICAL ANALYSIS

The epidemiologist (O.D.) in Canberra will examine associations statistically between MRS assessment and pathology and clinical variables using comparisons between means, contingency tables and logistic regression. Associations between MRS spectra and patient survival will be assessed by Kaplan - Meier Survival analysis (60) and proportional hazards regression models (61).

KEY RESEARCH ACCOMPLISHMENTS

- Proton Magnetic Resonance Spectroscopy (MRS) in conjunction with Statistical Computerised Strategies (SCS) provides an alternative modality for the pathological diagnosis and prognosis of breast fine needle aspiration (FNA) biopsies.
- Proton MRS on FNA distinguishes benign breast lesions from invasive cancer with a sensitivity and specificity of 95% and 96%, respectively.
- Proton MRS ranks carcinoma *in situ* specimens as benign or malignant.
- Spectra with a signal to noise ratios (SNR) > 25 are required to ensure a correlation of MR and histopathological data with a high sensitivity and specificity using visual inspection protocols.
- Multivariate analysis methods (Statistical Computerised Strategies, SCS) distinguishes benign and malignant breast lesions with high sensitivity and specificity including spectra with SNR<25.
- SCS predicts the presence or absence of vascular invasion and lymph node involvement for patients with invasive breast lesions.
- FNA breast specimens must be analysed by MRS within six months of collection to maintain the correlation with histopathological data.
- FNA specimens obtained through the skin generate MR spectra of adequate quality for analysis.
- Two dimensional MRS was unable to discriminate between breast tissues (due to large lipid contribution to the spectra) or breast FNAs (due to inadequate signal to noise).
- Proton MRS on FNA and Magic Angle Spinning (MAS) MRS detects metastatic disease in axillary lymph nodes from breast cancer patients.
- Proton MRS on FNA from sentinel lymph nodes from breast cancer patients generate MR data of adequate quality for SCS analysis.

REPORTABLE OUTCOMES

Manuscripts, Abstracts and Presentations;

1. Mackinnon WB, Barry PA, Malycha PL, Gillett DJ, Russell P, Lean, CL, Doran, ST, Barraclough BH, Bilous M, Mountford CE, FNB specimens of benign breast lesions distinguished from invasive cancer *ex vivo* with proton MRS, *Radiology*, 204:66, 1997.
2. Lo W, Lean CL, Dowd S, Malycha PL, Russell P and Mountford CE, Fine-needle biopsy of human lymph nodes. *Proc. Int. Soc. Mag. Res. Med.*, 1: 327, 1998.
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6. Lo W, Dowd S, Malycha P, Russell P, Mountford CE and Lean CL, Detection of Metastases in Lymph Nodes of Breast Cancer Patients Using Proton Magic Angle Spinning (MAS) MRS. *Proc. Int. Soc. Mag. Res. Med.*, 1: 451, 1999.
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8. Gluch L, Lean CL, Russell P, Malycha P, Mountford CE, Somorjai RL, Dolenko B, Nikulin A, Smith ICP. Pathology, Vascularisation and Nodal Involvement determined using Magnetic Resonance Spectroscopy from a Single Aspiration Breast Biopsy. Presented at: *The Australasian Society for Breast Disease*, Gold Coast, Australia, September, 1999.
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10. Lean CL, Russell P and Mountford CE. Magnetic Resonance Spectroscopy: A New Gold Standard for Medical Diagnosis. *Journal of Women's Imaging*, 2 (1): 19-30, 2000.
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Funding Applied For Based on Work Supported by the Award;

1. NHMRC, Clinical Centres of Excellence, 1998, 4 Years (Successful)
2. US Army Medical Defense and Materiel Command, Early Detection of Breast Cancer Using Magnetic Resonance Techniques, 2000, 4 years (Unsuccessful)
3. NHMRC, Detection of Metastases in Sentinel Nodes from Patients with Breast Cancer Using Proton MRS, 2000, 3 years (Successful)
4. Vera and Clive Ramaciotti Foundation, *In Vivo* Breast Coil, 2000, 1 year (Successful)
5. NHMRC, Application of MRI and MRS to the Diagnosis and Staging of Breast Cancer, 2001, 3 years (Pending)

CONCLUSIONS 1997/98

- This project is proceeding as outlined in the original document and as described above.
- Of the three "purposes" listed on page 6 all three are underway.
- Hypotheses 1 and 2 (page 6) have been shown to be correct. Data collected so far indicate that hypothesis 4 is also correct.
- No unforeseen difficulties have been encountered and the program is proceeding to schedule.

CONCLUSIONS 1998/99

- The project has proceeded as outlined in the original document with the addition of MRS detection of metastatic breast cancer in the axillary lymph nodes of breast cancer patients. Accurate identification of lymph node metastases is of primary importance in the staging and therefore management of breast cancer patients.
- All three purposes listed on page 7 are underway.
- Hypotheses 1 and 2 have now been shown to be correct by both visual inspection and CCD (multivariate analysis) methods. Additional data support hypothesis 4 also being correct.
- Difficulties that have been encountered include:
 - Small numbers of lobular cancers available

- Problem of incomplete water suppression affecting multivariate analysis methods.
Difficulty overcome with new pulse sequences.
- Signal to noise ratio affecting sensitivity and specificity of visual inspection method.
This was overcome using CCD (multivariate analysis) methods.

CONCLUSIONS 1999/2000

- The project has proceeded as outlined in the original document with the addition of MRS detection of metastatic breast cancer in the axillary and sentinel lymph nodes of breast cancer patients. Accurate identification of metastases in lymph nodes, and in particular sentinel lymph nodes, is of primary importance in the staging and therefore management of breast cancer patients.
- Hypotheses 1 and 2 have now been shown to be correct by both visual inspection and SCS (multivariate analysis) methods.
- Data support hypothesis 4 being correct. SCS analysis will be undertaken when 30 specimens of each of MRS malignant DCIS and MRS benign DCIS are obtained.
- Difficulties that have been encountered include:
 - Small numbers of lobular cancers, breast hyperplasia and normal breast tissues.
 - Problem of incomplete water suppression affecting multivariate analysis methods.
Difficulty overcome with new pulse sequences.
 - Signal to noise ratio affecting sensitivity and specificity of visual inspection method.
This was overcome using SCS (multivariate analysis) methods.

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PUBLICATIONS/MEETING ABSTRACTS/PAID PERSONNEL

PUBLICATIONS:

1. Mackinnon WB, Barry PA, Malycha PL, Gillett DJ, Russell P, Lean, CL, Doran, ST, Barraclough BH, Bilous M, Mountford CE, FNB specimens of benign breast lesions distinguished from invasive cancer *ex vivo* with proton MRS, *Radiology*, 204:66, 1997.
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MEETING ABSTRACTS:

1. Lo W, Lean CL, Dowd S, Malycha PL, Russell P and Mountford CE, Fine-needle biopsy of human lymph nodes. *Proc. Int. Soc. Mag. Res. Med.*, 1: 327, 1998.
2. Himmelreich U, Mackinnon WB, Barry P, Russell P, Neilson G and Mountford CE. Proton MR for Pathological Cancer Diagnosis in Fine Needle Biopsy Specimen of Breast Cancer Lesions. *Proc. Int. Soc. Magn. Res.*, 788-789, 1998.
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PAID PERSONNEL

- | | | |
|-----|-------------------------|--------------------------------|
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| 2. | Professor Peter Russell | Pathologist |
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| 7. | Dr Russell Banks | Information Technology Support |
| 8. | Ms Sinead Doran | Spectroscopist |
| 9. | Ms Agata Rekas | Spectroscopist |
| 10. | Ms Rebecca Hancock | Administrative Support |
| 11. | Ms June Watzl | Technical Support |

APPENDIX I

ACRONYM /SYMBOL DEFINITION

1D	-	one dimensional
2D	-	two dimensional
¹H	-	proton
12p	-	chromosome 12 p (short) arm
18q	-	chromosome 18 q (long) arm
CA125	-	tumour marker
CA153	-	tumour marker
cERB2	-	tumour marker
CH/CH₂	-	methine to methylene ratio
CH₂/CH₃	-	methylene to methyl ratio
CIN	-	cervical intraepithelial neoplasia
CINI	-	cervical intraepithelial neoplasia - stage I
CINII	-	cervical intraepithelial neoplasia - stage II
CINIII	-	cervical intraepithelial neoplasia - stage III
CIS	-	carcinoma <i>in situ</i>
CPMG	-	Carr-Purcell-Meiboom-Gill
COSY-		COrelated SpectroscopY
CW	-	continuous wave
dB	-	decibel
D₂O	-	deuterium oxide
ER	-	oestrogen receptor
f₁ and f₂	-	frequency in the first and second dimensions of a 2D MR experiment
FAA	-	formalin:acetic acid:alcohol
FID	-	free induction decay
FNB	-	fine needle biopsy
Fuc	-	fucose
GB	-	Gaussian broadening
G-CSF	-	granulocyte colony stimulating factor

H₅-H₆	-	coupling between protons attached to the C ₅ and C ₆ of carbohydrate moieties
Hz	-	Hertz
K-<i>ras</i>	-	oncogene
LB	-	Lorentzian broadening
Le^y	-	Lewis ^y (antigen)
MAS	-	Magic Angle Spinning
MHz	-	mega Hertz
MRI	-	magnetic resonance imaging
MRS	-	magnetic resonance spectroscopy
MR	-	magnetic resonance
NRC	-	National Research Council of Canada
p53	-	tumour supressor gene
PABA	-	p-aminobenzoic acid
PBS	-	phosphate buffered saline
PCA	-	principal component analysis
ppm	-	parts per million (units of chemical shift)
PR	-	progesterone receptor
SFNAB	-	stereotactic fine needle aspiration biopsy
SCS	-	statistical computerised strategies
t₁ and t₂	-	first and second time domains in a 2D MR experiment
T1	-	tumour size less than 2 cm
T2	-	tumour size 2 - 5 cm
T3	-	tumour size greater than 5 cm
T₂	-	spin-spin (transverse) relaxation
Thr/Fuc	-	threonine/fucose
TIL	-	tumour infiltrating lymphocytes ¹² .

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Fine-Needle Biopsy Specimens of Benign Breast Lesions Distinguished from Invasive Cancer ex Vivo with Proton MR Spectroscopy¹

PURPOSE: To determine whether invasive breast cancer can be distinguished from benign lesions with proton magnetic resonance (MR) spectroscopy ex vivo on the basis of altered cellular chemistry.

MATERIALS AND METHODS: Two hundred eighteen fine-needle biopsy specimens were obtained in 191 patients undergoing surgery and were analyzed with proton MR spectroscopy. MR spectroscopic and histopathologic findings were compared.

RESULTS: Invasive carcinoma produced increased signal at 3.25 ppm, attributable to choline-containing metabolites. Discrimination between invasive carcinoma ($n = 82$), benign lesions ($n = 106$), or carcinoma in situ ($n = 17$) was based on the resonance intensity at 3.25 ppm standardized to the resonance at 3.05 ppm ($P < .001$). The ratio of peak height intensities of resonances at 3.25 to those at 3.05 ppm was less than 1.7 in 102 of the 106 normal or benign lesions. All carcinoma in situ specimens with comedonecrosis or a microinvasive component ($n = 6$) were categorized at MR spectroscopy with invasive carcinoma, while others with in situ disease alone were categorized with benign lesions ($n = 11$). The sensitivity and specificity of MR spectroscopy in fine-needle biopsy specimens in distinguishing benign lesions from invasive cancer were 95% and 96%, respectively.

CONCLUSION: Proton MR spectroscopy of fine-needle biopsy specimens provides objective diagnostic information that complements findings of conventional preoperative investigations of breast lesions.

IN Australia, breast cancer is the leading cause of cancer-related death in women (1). The incidence of breast cancer outranks all other cancers in women older than 35 years. In the past decade, the incidence has risen by 25%, and the lifetime risk (from birth to the age of 74 years) of breast cancer in white women is comparable to Western world figures at one in 13 (1).

Recent improvement in breast cancer patient outcome is due to earlier diagnosis and more effective management (2,3). The combination of physical examination, mammography, and fine-needle aspiration cytologic analysis (triple assessment) is to date the most sensitive method for the preoperative diagnosis of clinically and radiographically detected breast lesions. While triple assessment has a high probability of detection of all malignant lesions, the suboptimal specificity results in diagnostic uncertainty; thus, open biopsy is needed to exclude malignancy in many women.

Physical examination has limitations due to individual variation in breast consistency, the site and size of the lesion (lesions smaller than 1 cm are usually impalpable), and the presence of a diffuse versus discrete tumor. Screening mammography guidelines ensure that biopsy is performed in approximately one benign lesion

for every malignant lesion detected (4). Even so, 10%–40% of palpable breast cancers are missed at mammography alone, especially in women younger than 50 years in whom radiographically opaque breast tissue may obscure changes associated with malignancy (5–8). Fine-needle aspiration cytologic analysis has a sampling error rate in the range of 1%–15% (9), which partly explains the varied sensitivity. While the complete sensitivity of fine-needle aspiration biopsy is 81%–97% (10), this includes the 50%–80% of cases with atypical and suspicious diagnoses in which cancer is typically confirmed histologically with open biopsy (11).

A technology that monitors cellular chemistry, which correlates with different cell behaviors, could offer both independent and objective assessment of breast tissue. The potential then would exist to identify a predisposition toward or early features of breast cancer and offer interventions aimed at reducing tumor development. Proton magnetic resonance (MR) spectroscopy is one diagnostic modality that has been successfully applied to monitor tumor development and progression in other organs (12–18). Pre-invasive cancer of the uterine cervix can be distinguished from invasive cancer by means of proton MR spectroscopy, with a sensitivity and speci-

Index terms: Breast, biopsy, 00.1261, 00.1262 • Breast neoplasms, diagnosis, 00.30 • Breast neoplasms, MR, 00.12145, 00.30 • Magnetic resonance (MR), spectroscopy, 00.12145

Abbreviation: DCIS = ductal carcinoma in situ.

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ficity of 98% and 94%, respectively (13). The technique also distinguishes between genuinely benign and malignant follicular lesions in the human thyroid (18) and helps discriminate between degrees of loss of cellular differentiation in ovarian tumors (16).

Preliminary unlocalized one- and two-dimensional proton MR spectroscopic examinations of excised breast tissue showed increased levels of glycerophosphocholine and phosphocholine in invasive breast carcinoma compared with levels in benign fibroadenomas (19). Specimens of benign fibrocystic disease were characterized by an absence of resonances from choline, amino acids, and other metabolites. However, the high adipose content in excised breast tissue resulted in major difficulty in attaining adequate spectral resolution. The intense broad MR resonances from this fat often masked other diagnostic resonances in the one-dimensional spectrum. This problem is largely overcome by applying a T2 filter (Bruker, Karlsruhe, Germany; Carr-Purcell-Meiboom-Gill sequence) during data collection (20), postacquisitional data processing, or both (21). A simpler remedy was to optimize specimen collection. Collection procedures substantially affected the amount of exogenous fat sampled. Fine-needle aspiration biopsy methods developed for thyroid sampling (17) provided cellular material adequate for assessment with MR spectroscopy with fat levels lower than those in excised tissue.

Our objectives for this study were (a) to assess the sensitivity and specificity of proton MR spectroscopy in fine-needle biopsy specimens for delineating invasive breast cancer *ex vivo*, (b) to determine whether carcinoma *in situ* can be distinguished from invasive carcinoma, and (c) to compare diagnosis with MR spectroscopy in fine-needle biopsy specimens with diagnosis with traditional preoperative investigations such as physical examination, mammography, and fine-needle aspiration cytologic analysis.

MATERIALS AND METHODS

All histopathologic and MR spectroscopic analyses were blind. MR spectroscopic data were correlated with clinicopathologic criteria after all reports were filed.

Patients

Two hundred eighteen fine-needle biopsy or tissue specimens for MR spectroscopic analysis or correlative histopatho-

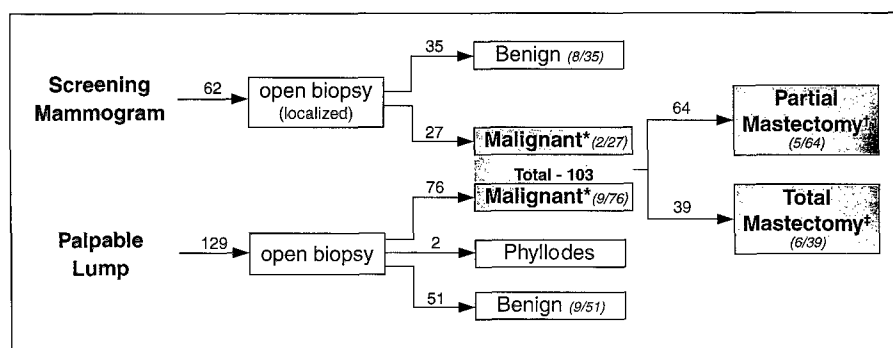


Figure 1. Flow chart for management and final histopathologic diagnosis. The numbers in parentheses indicate the numbers of patients who had an uncertain cytologic diagnosis during the diagnostic work-up. The numbers alongside the arrows indicate the total numbers of patients. Specimens obtained in 11 patients were rejected. * = invasive and *in situ* carcinoma, † = lumpectomy and quadrantectomy (nine of these patients underwent complete mastectomy [not shown]), ‡ = 12 for *in situ* carcinoma and 27 for invasive carcinoma.

logic analysis, respectively, were obtained in 191 consecutive patients undergoing diagnostic biopsy or definitive treatment (lumpectomy, quadrantectomy, or mastectomy) for histologically proved invasive breast cancer. Indications for surgery included mammographically detected palpable lesions, as well as palpable mass lesions, which had suspicious mammographic, fine-needle aspiration cytologic analysis, or clinical examination findings (Fig 1). The age range of patients was 20–81 years (mean age \pm standard deviation, 52 years \pm 14). When mastectomy for invasive carcinoma was performed, control specimens of macroscopically uninvolved breast tissue (which was later confirmed histologically) were obtained in the same patient ($n = 27$).

Specimen Collection

Previous findings of MR spectroscopic analysis in fine-needle biopsy thyroid specimens had established that as few as 10^6 cells are required to obtain one-dimensional MR spectroscopic data with an adequate signal-to-noise ratio in less than 15 minutes (256 accumulations) (17). To acquire an adequate breast specimen, fine-needle biopsy was performed with multiple (typically six) passes with a 23-gauge needle either through the resected lesion *ex vivo* ($n = 129$) or *in vivo* after lesion identification during open biopsy ($n = 89$). These techniques could be guaranteed to provide cells from the lesion and, in addition, allowed the aspiration site to be identified at excision. Tissue from the aspiration site (3 mm³) was collected for correlative histopathologic analysis.

Specimen Handling

Cells or tissue specimens were placed in polypropylene vials that contained 300 μ L of phosphate-buffered saline in deuterium oxide. All specimens were immediately immersed in liquid nitrogen and stored at -70°C for up to 6 weeks until MR spectroscopic analysis.

Preparation of Specimens for Proton MR Spectroscopy

Before proton MR spectroscopy, each fine-needle biopsy specimen was thawed and transferred directly to a 5-mm MR spectroscopic tube. The volume was adjusted to 300 μ L with phosphate-buffered saline in deuterium oxide when necessary. The sample tube was fitted with a capillary insert that contained 60 μ L of paraaminobenzoic acid (10 mmol/L in the phosphate-buffered saline–deuterium oxide solution) as an external standard.

Proton MR Spectroscopy

Proton MR spectroscopic assessment of all specimens was performed without knowledge of the final histologic diagnosis.

Data acquisition.—MR spectroscopic experiments were performed at 360 MHz or 8.5 T with a wide-bore spectrometer (model AM-360; Bruker) that was equipped with a computer (model 3000; Aspect, Karlsruhe, Germany) and a standard 5-mm dedicated proton probe head (Bruker). The sample was spun at 20 Hz, and the temperature was maintained at 37°C . Residual water signal was suppressed with selective gated irradiation by using low power (15 dB below 0.2 W). The chemical shifts of resonances were referenced to aqueous sodium 3-(trimethylsilyl)propanesulphonate at 0.00 ppm. One-dimensional spectra were acquired as previously described (19) over a sweep width of 3,597 Hz (10.0 ppm) by using a 90° pulse, 8,192 data points, 256 free induction decays, an acquisition time of 1.14 seconds, and a relaxation delay of 2.00 seconds, which resulted in a pulse repetition time of 3.14 seconds.

Data processing.—Data processing for each specimen was performed independently by two operators (P.A.B., S.T.D.) without access to the histopathology reports; one used the spectrometer's computer (model 3000; Aspect), and the other used a Unix workstation (model X32; Bruker). A line broadening of 3.0 Hz was applied to the data before Fourier transform.

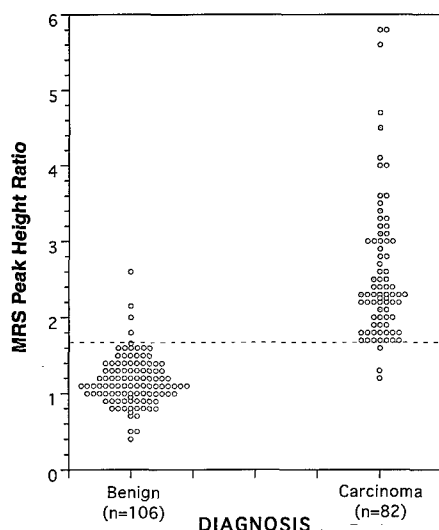


Figure 2. Breast fine-needle biopsy MR spectroscopic (MRS) findings of unequivocally benign versus infiltrating carcinoma. MR spectroscopic ratios determined as shown in Figure 3. Data are grouped on the basis of the final histopathologic findings in tissue specimens from the aspiration site.

Table 1
Summary of Histologic Subtypes of Fine-Needle Biopsy Specimens

Histopathologic Subtype	No. of Lesions
Benign (<i>n</i> = 106)	
Fibrocystic changes	68
Fibroadenoma	15
Ductal hyperplasia (mild to florid)	9
Fat necrosis	4
Sclerosing adenosis	2
Radial scar	2
Atypical ductal hyperplasia	2
Ductal ectasia	2
Miscellaneous	2
Phyllodes tumor	2
DCIS* (<i>n</i> = 17)*	
High grade	10
Low grade	1
Comedonecrosis	2
With microinvasion	4
Invasive carcinoma (<i>n</i> = 82)	
Ductal carcinoma of no special type	66
Ductal carcinoma of no special type with an extensive intraductal component	7
Lobular carcinoma	5
Tubular carcinoma	4

Note.—Histopathologic findings are the dominant findings in correlative histopathologic samples.

* DCIS = ductal carcinoma in situ.

mation. Zero- and first-order phase corrections were used in the data. Baselines were corrected by using a fourth-order polynomial baseline-correction routine.

Data analysis.—The ratio of the peak height intensities of spectral resonances at 3.25 ppm to those at 3.05 ppm (the MR spectroscopic ratio) was calculated by each operator, and the two results for each speci-

men were averaged. This ratio was used to generate Figure 2. Data were rejected because of an inadequate signal-to-noise ratio (in 11 specimens) if the peak intensity of the CH₂ resonance at 1.3 ppm in the fine-needle biopsy specimen spectrum was less than 40% of the intensities of those spectra generated by the external standard or if the calculated signal-to-noise ratio was less than 25.

Histopathologic Analysis

Diagnostic correlation was attained by comparing the diagnosis made with the MR spectroscopic ratio with the postoperative hospital histopathologic report in each patient. The final histologic diagnoses in the study group are shown in Table 1.

Without reference to clinical or MR spectroscopic data, cytologic analysis of the aspirate after MR spectroscopic analysis was attempted. However, cellular detail was compromised by autolytic changes, and this approach was not pursued. Therefore, to verify fine-needle biopsy sampling accuracy, a single pathologist (P.R.) separately performed histopathologic assessment in tissue specimens removed from the aspiration sites of the MR spectroscopic specimens (see Specimen Collection). These tissue specimens were thawed; fixed in a mixture of formalin, acetic acid, and alcohol; embedded in paraffin; sectioned at 7 μ m; stained with hematoxylin and eosin according to standard protocols; and reviewed by the pathologist under the light microscope without access to the clinical or MR spectroscopic data. Tissue preservation, the abundance of epithelial cells relative to stroma, and the presence of potentially confounding factors such as fat and inflammatory cells were reported in addition to the principal diagnosis.

Other Clinical Correlations

Comparisons were made with findings of other preoperative diagnostic investigations, which included physical examination, mammography, and fine-needle aspiration cytologic analysis (separate specimen than that in MR spectroscopy).

RESULTS

Sample Collection

The problem of high levels of MR-measurable fat in the solid breast tissue was successfully overcome with fine-needle aspiration biopsy. The resultant improvement in spectral resolution due to the reduction in exogenous fat was confirmed by means of histopathologic analysis (data not shown).

In 207 of the 218 fine-needle biopsy specimens obtained, sufficient cellular material was collected for adequate MR assessment under the chosen ex-

perimental conditions. Eleven specimens were discarded for technical reasons (see Materials and Methods, Proton MR Spectroscopy, Data analysis).

Benign Lesions versus Invasive Carcinoma

Invasive carcinoma was identified by means of resonances at 3.25 ppm that were attributable to choline-containing metabolites (Fig 3). A discrimination between invasive carcinoma (*n* = 82) and normal or benign tissue (*n* = 106) was made on the basis of the intensity of the 3.25 ppm resonance standardized to the resonance intensity at 3.05 ppm, indicating contributions from creatine, phosphocreatine, or lysine (*P* < .001, Mann-Whitney *U* test) (Fig 2). A receiver operating characteristic curve calculated by using this intensity ratio is shown in Figure 4.

Of 106 benign or normal samples, 102 had an MR spectroscopic ratio of less than 1.7. Four false-positive findings were obtained in three palpable fibroadenomas (fine-needle aspiration cytologic analysis had been performed in none of them) and in one moderate ductal hyperplastic lesion. The diagnoses were based on correlative histopathologic findings.

Fine-needle biopsy in four of 82 carcinomas had an MR spectroscopic ratio of less than 1.7. Correlative histopathologic findings in specimens from the aspiration site showed that one specimen had only benign fibrocystic changes in this region. The other three specimens were confirmed as invasive carcinoma, but with a marked inflammatory cell infiltrate.

Two specimens with phyllodes tumors were studied. The first was multifocal and had a ratio of more than 1.7, and the second was from a single mass and had a ratio of 1.6. Phyllodes tumors are classified as fibroepithelial proliferations with variable biologic activity. Their behavior ranges from benignity through a propensity to local recurrence to blood-borne metastasis. Both phyllodes tumors were excluded from all statistical analyses.

Carcinoma in Situ

The MR spectroscopic ratios for all samples reported as DCIS on routine hospital histopathology reports are shown in Figure 5. These were grouped according to the correlative histopathologic findings. No in situ disease was detected in the correlative histopathologic analysis of the six samples denoted "benign." Ductal cells had breached the basement membrane

(<1 mm) at one or more foci in the entirety of each of the four DCIS specimens denoted "microinvasion." This group, as well as two specimens of high-grade DCIS with extensive comedonecrosis had ratios of more than 1.7. Specimens that contained only DCIS (10 high grade, one low grade) all had ratios of at most 1.7, which is indicative of a low ratio of choline to creatine and lysine, similar to that in benign lesions.

Clinical Correlations

All cases that were mammographically suspicious ($n = 56$), mammographically negative ($n = 23$), or nondiagnostic ($n = 14$) or that had atypical or suspicious fine-needle aspiration biopsy cytologic findings ($n = 25$) were accurately categorized at MR spectroscopy as benign or malignant, as confirmed with histopathologic analysis of tissue excised from the aspiration site.

MR spectroscopic findings in fine-needle biopsy specimens correlated with the final histologic diagnosis of benign lesions in 96% of cases (Table 2); yet, biopsy was performed because of clinical (34%) or mammographic features (45%) or because of fine-needle aspiration biopsy cytologic analysis results (31%). MR spectroscopic findings in fine-needle biopsy specimens correlated with a malignant histologic diagnosis in 95% of cases (Table 2). While biopsy for all the malignant lesions studied was indicated with the triple assessment, no single preoperative modality was an improvement on MR spectroscopy in the identification of malignancy (physical examination, 84%; mammography, 82%; fine-needle aspiration cytologic analysis, 92%).

DISCUSSION

When compared with the histopathologic analysis, the peak intensity ratio in MR spectroscopy of fine-needle biopsy specimens has a sensitivity and specificity for the differentiation of invasive carcinoma from benign breast lesions of 95% and 96%, respectively. The increase in the ratio observed in carcinoma tissues is most likely due to elevated choline metabolite levels in malignant compared with benign tissues. This is consistent with a higher rate of cellular replication, specifically with increased phospholipid synthesis and membrane turnover (12).

Although triple assessment followed by open biopsy has an overall sensitivity that approaches 100%, it

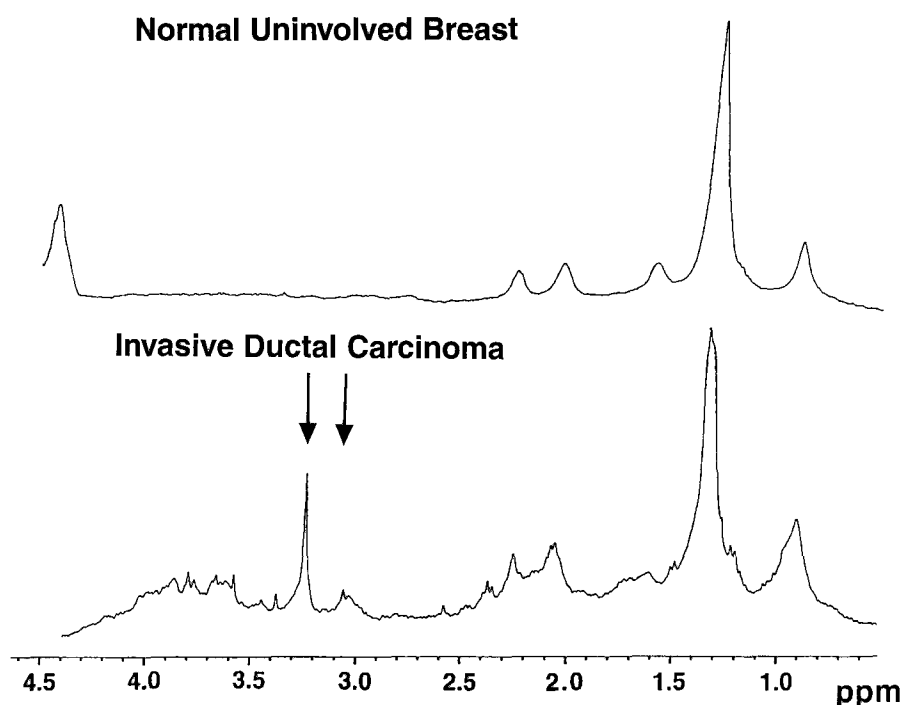


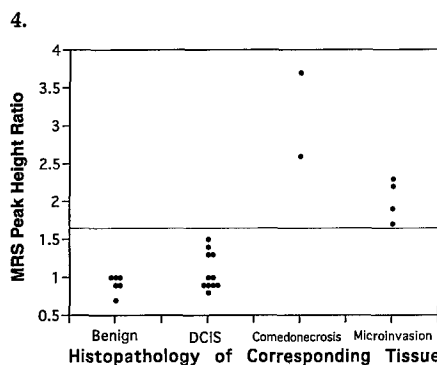
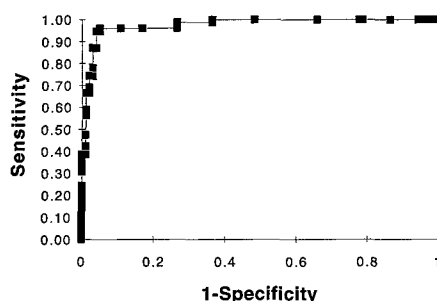
Figure 3. One-dimensional proton MR spectra (256 acquisitions) in fine-needle biopsy specimens obtained in a patient who underwent mastectomy. Spectra for a normal uninvolved breast and a breast with invasive ductal carcinoma. The distinction between normal breast and invasive carcinoma is based on an increase in the *N*-trimethyl resonance at 3.25 ppm normalized to that of creatine at 3.05 ppm (peaks indicated by arrows).

entails a large number of open biopsies for benign disease. This is especially the case when equivocal or suspicious mammographic findings are followed by either atypical or suspicious fine-needle aspiration cytologic or benign cytologic findings that are incongruous with clinical or radiologic findings. In our study, all atypical or suspicious fine-needle aspiration cytologic results in subsequently confirmed benign specimens had a benign MR spectroscopic ratio. While the MR spectroscopic findings were obtained in ex vivo aspirate, MR spectroscopy performed before cytologic analysis in in vivo aspirates may well be a valuable adjunct because it improves the specificity. Therefore, MR spectroscopy in fine-needle biopsy specimens could reduce the number of biopsies performed in benign lesions and could lead to a more conservative approach, such as continued observation or surveillance with repeat fine-needle aspiration cytologic analysis and MR spectroscopy. Used as a complementary modality to triple assessment, MR spectroscopy in fine-needle biopsy specimens may also provide further diagnostic confidence when open biopsy is requested for patient reassurance only.

MR spectroscopy in fine-needle biopsy specimens clearly distinguished pure DCIS without comedonecrosis or

microinvasion (MR spectroscopy ratio, ≤ 1.7) from invasive carcinoma. However, even when DCIS specimens contained comedonecrosis or a few foci of microinvasion, the specimen was categorized in the invasive category with MR spectroscopy in every case. This may demonstrate the selective sampling of necrotic cells or invasive disease in the MR spectroscopic fine-needle biopsy specimens but could also indicate chemical changes that occur in cells that progress from in situ to frankly invasive before morphologic manifestation. Pure DCIS was not distinguished from benign lesions on the basis of the MR spectroscopic ratio; however, more spectral information is available for comparison of the two groups, and this is being investigated. In particular, specific chemical differences characteristic of pure DCIS may be identified with two-dimensional MR spectroscopy. Alternatively, multivariate analysis as previously reported for thyroid neoplasms (22), yet to be undertaken, is likely to improve sensitivity and specificity.

Seventy-eight of the 82 invasive carcinomas had an MR spectroscopic ratio of greater than 1.7. Of the four false-negative specimens, one aspiration sample was found not to contain invasive carcinoma. The remaining three samples all had a marked inflam-



Figures 4, 5. (4) Receiver operating characteristic curve of the data from Figure 2 ($n = 188$) calculated from the MR spectroscopic ratios of all specimens. The ratios were ranked, and both the sensitivity and the specificity were calculated for all ratios. (5) MR spectroscopic (MRS) ratios in fine-needle biopsy specimens reported as containing DCIS ($n = 23$) at postoperative histologic analysis. Data are grouped on the basis of the final correlative histopathologic findings.

matory infiltrate, which, if sampled, may have accounted for a ratio of less than 1.7. In all the remaining carcinoma specimens, which includes those that were mammographically negative or had nondiagnostic fine-needle aspiration cytologic analysis results, MR spectroscopy correctly indicated malignancy.

In this study, fine-needle biopsy specimens for MR spectroscopy were collected intraoperatively from the exposed lesion to ensure accurate sampling. If MR spectroscopy in fine-needle biopsy specimens were used as a preoperative diagnostic modality, a sampling error could be introduced, similar to that experienced with fine-needle aspiration cytologic analysis, which would reduce the sensitivity and specificity recorded here. For example, in this patient sample, 14 of 143 cytology reports were nondiagnostic or insufficient. Further studies will directly compare MR spectroscopy with cytology in the same aspirate at the time of clinical assessment.

The potential clinical use of MR spectroscopy in fine-needle biopsy specimens is to complement triple assessment procedures, which would

Table 2
Diagnosis with MR Spectroscopy of Fine-Needle Biopsy Specimens Compared with Histopathologic Diagnosis of Tissue Samples

MR Spectroscopic Finding	Histopathologic Finding (N = 188)	
	Invasive Carcinoma	Normal or Benign
Positive*	78	4
Negative†	4	102

Note.—The sensitivity of MR spectroscopy was 95% (78 true-positive findings of 82 total findings), the specificity was 96% (102 true-negative findings of 106 total findings), the positive predictive value was 95% (78 true-positive findings of 82 total findings), and the negative predictive value was 96% (102 true-negative findings of 106 total findings).

* MR spectroscopic ratio of at least 1.7.

† MR spectroscopic ratio of less than 1.7.

reduce the number of unnecessary biopsies in benign lesions. Management of breast lesions varies, however, from country to country. In Australia, fine-needle biopsy is routinely used as part of the triple assessment; thus, introducing MR spectroscopy as a fourth modality would not alter management. In the United States, core biopsy is used more often. Core biopsy has two potential areas of usefulness, namely, to demonstrate an invasive focus within an area of DCIS and to histologically confirm radiologically suspected benign lesions. It is anticipated that MR spectroscopy in fine-needle biopsy specimens will be able to address both these questions as or more accurately than core biopsy, which would obviate the more invasive procedure. A core biopsy specimen may contain high levels of fat, which makes it unsuitable for MR spectroscopic analysis. Thus, in countries such as the United States, the choice of biopsy procedure will need to be reassessed and the re-introduction of fine-needle biopsy will need to be measured against MR spectroscopy, with the improved sensitivity and specificity.

Furthermore, it is likely that the technical obstacles to in vivo spectroscopy of the breast will be overcome. It has been shown in at least one U.S. site that the diagnostic chemical information reported here is available in vivo. Considerable research and development are required, however, before such techniques will be in routine clinical use.

MR spectroscopy in ex vivo fine-needle biopsy specimens may (a) complement triple assessment and reduce

the number of performances of unnecessary biopsy of benign lesions and (b) obviate open biopsy before the definitive therapy of invasive lesions by increasing the diagnostic specificity of the cytologic analysis. Preoperative diagnosis of breast lesions with proton MR spectroscopy thereby offers potential benefits in management by reducing the potential morbidity related to biopsy and by allaying anxiety due to an equivocal diagnosis. ■

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CONTINUING MEDICAL EDUCATION

MAGNETIC RESONANCE SPECTROSCOPY AND BREAST CANCER

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Key words: breast cancer, fine needle aspiration cytology, magnetic resonance, tumour development.

WHAT IS MAGNETIC RESONANCE?

The magnetic resonance (MR) experiment involves the excitation of a nuclear spin system. After excitation the system returns to equilibrium and depending on the type of experiment, several types of phenomena can be measured providing chemical and/or spatial information. Different nuclei can be examined (e.g. ^{31}P , ^1H and ^{13}C) but the vast majority of medical MR applications observe the proton (^1H) nucleus. The sample assessed by MR may be a fine needle aspiration (FNA) biopsy, a tissue biopsy or a whole patient. If one considers the many protons in a single cell, not to mention an entire organ, the need for computational assistance in collecting and processing the MR data becomes clear.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) gives rise to computer-generated images of water protons within the body which provide detailed spatial information. The MR data, collected at the single frequency of water protons, are reconstructed into an image that allows anatomy and disease to be identified simultaneously. However, water-based MRI techniques are not able to provide the pathological diagnosis offered by current histological or microbiological techniques. For example, the size and position of a brain lesion can be identified by MRI but a biopsy is usually required to distinguish tumour from infection.

Magnetic resonance spectroscopy

Magnetic resonance spectroscopy (MRS) measures protons over a range of frequencies, thus collecting information on a large number of chemical species (including water) present in the sample. The water signal is usually predominant and must therefore be suppressed in order to record chemicals present in much smaller quantities. The basis of MRS is molecular motion. Thus, only chemicals present in reasonable concentrations and mobile on the MR time scale will be recorded.

The magnet in which the data are collected also varies and determines the type of experiment performed. For larger specimens, for example a human body, the bore of the magnet needs to be approximately 60 cm. The strength of the magnet for whole-body MRI is usually 1.5 Tesla, although strengths as high as 4 Tesla whole-body systems are available. For imaging purposes 1.5 Tesla is adequate. However, for spectroscopy, higher field strengths allow greater separation of the many measurable

chemical species and thus for human *in vivo* spectroscopy a 3 or 4 Tesla magnet is of great benefit. Tissue biopsies examined *ex vivo* are assessed using a magnet strength of 8 Tesla with a narrow bore of 10 cm.

HISTORY AND RECENT DEVELOPMENT OF MRS

Magnetic resonance spectroscopy has been used by biochemists for over 30 years for the evaluation of cellular chemistry. Magnetic resonance technology was applied to medical diagnoses with the introduction of MRI. This effectively bypassed spectroscopy, the original science from which MRI was developed. The early experiments using proton MRS for cancer diagnoses were not reproducible. It was originally thought that it was the spectroscopy method at fault. We now know that the chemistry of cancer cells changes from the time the cell ceases to be normal until the patient dies from secondary disease and that the MRS was correctly reporting this ongoing change.

The 'gold standard' for cancer diagnosis, histopathology, relies on alterations to cell morphology and tissue architecture to make a diagnosis. It is clear from retrospective studies of clinical outcome that diagnostic information is being missed when using currently available techniques. Magnetic resonance studies undertaken in Australia over the last two decades have been aimed at addressing this problem, the premise being that diseased cells have pools of chemicals measurable by MRS that are both diagnostic and prognostic. By reporting upon the chemical status of the cell at each stage of tumour development, a temporal study is obtained which records early variations that are not morphologically manifest and thus not detectable by light microscopy. The potential then exists to identify a predisposition towards or the early features of cancer and offer intervention aimed at reducing tumour development.

The pioneering work to assess MRS in the characterization of tumour development and progression was originally undertaken in the Department of Cancer Medicine, University of Sydney and is now under way at the Institute for Magnetic Resonance Research (IMRR), established in 1995. Pathologists and surgeons have provided vast quantities of clinical material, the characterization of which has led to a new MRS-based medical science. To date cervix, colon, ovary, lung, thyroid, lymph nodes, oesophagus, prostate, brain and, of course, breast have been studied.

Two consistent outcomes from these research programmes, irrespective of which organ has been studied, are:

1. Invasive biopsies can be distinguished from benign and healthy tissues with a sensitivity and specificity approaching 99%.
2. Pre-invasive states that are morphologically benign and indistinguishable under the light microscope have different chemical fingerprints as measured by MRS.

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Some histologically benign lesions have been identified by MRS as having a chemical profile closer to malignancy than benign. These were all in patients with malignant disease elsewhere in the same organ, indicative of a commitment to malignancy.

BREAST CANCER

Over 250 breast patients took part in the first study.¹ Fine needle aspiration biopsies were taken from the breast and examined

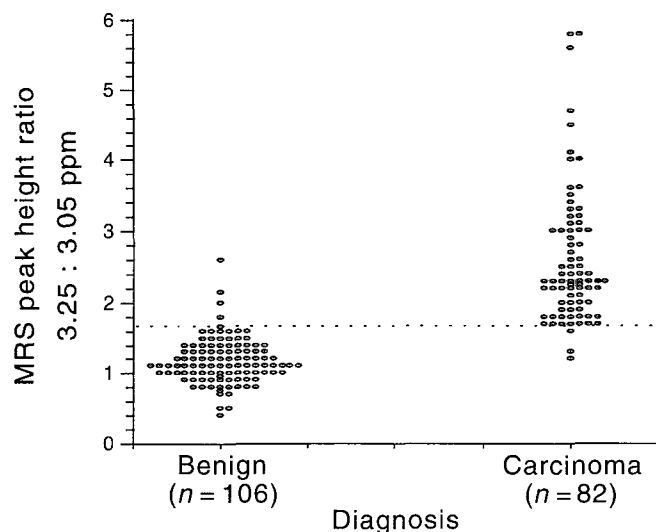


Fig. 1. One-dimensional magnetic resonance spectroscopy (8.5 Tesla) obtained from fine needle biopsy specimens from patients who underwent mastectomy. Spectra for normal non-involved breast and from invasive ductal carcinoma. The distinction between normal breast and invasive carcinoma is based upon an increase in the N-trimethyl resonance at 3.25 ppm normalized to that of creatine at 3.05 ppm.

by MRS and then conventional histology. Comparisons were made with other pre-operative diagnostic investigations including physical examination, mammography and FNA cytology.

Invasive cancer was identified by the presence of choline-containing metabolites (Fig. 1). Magnetic resonance spectroscopy discriminated between invasive cancer and normal or benign tissue based on the amount of choline when standardized to the creatine, phosphocreatine and lysine present in the sample (Fig. 2). Magnetic resonance spectroscopy on FNAB when compared with the histopathological diagnosis has a sensitivity and specificity for the distinction of invasive carcinoma from benign breast lesions of 95% and 96%, respectively. These data have now been sent to the National Research Council of Canada where a team of mathematicians will produce an algorithm to allow a robust, non-subjective diagnosis to be made which is independent of human involvement.

All samples reported as ductal carcinoma *in situ* (DCIS) by routine hospital histopathology ($n = 21$) were grouped according to the correlative histopathology on the MRS specimen. Magnetic resonance spectroscopy subcategorized DCIS into two categories. In category A (high choline to creatine/lysine ratio) there were four DCIS samples where ductal cells had breached the basement membrane (< 1 mm) in one or more foci and four samples where micro-invasion was identified. These eight samples as well as two samples of high-grade DCIS with extensive comedo necrosis were malignant by MR criteria. Group B (low choline to creatine/lysine ratio) contained 10 high-grade and one low-grade DCIS; all had MR profiles similar to that obtained for benign lesions.

Thus MRS on FNAB clearly distinguished pure DCIS (without comedo necrosis or micro-invasion) from invasive carcinoma. However, when DCIS specimens contained comedo necrosis or minute foci of micro-invasion undetected by histopathology, MRS ranked the specimen in the invasive category in every case. This indicates that chemical changes are occurring in cells

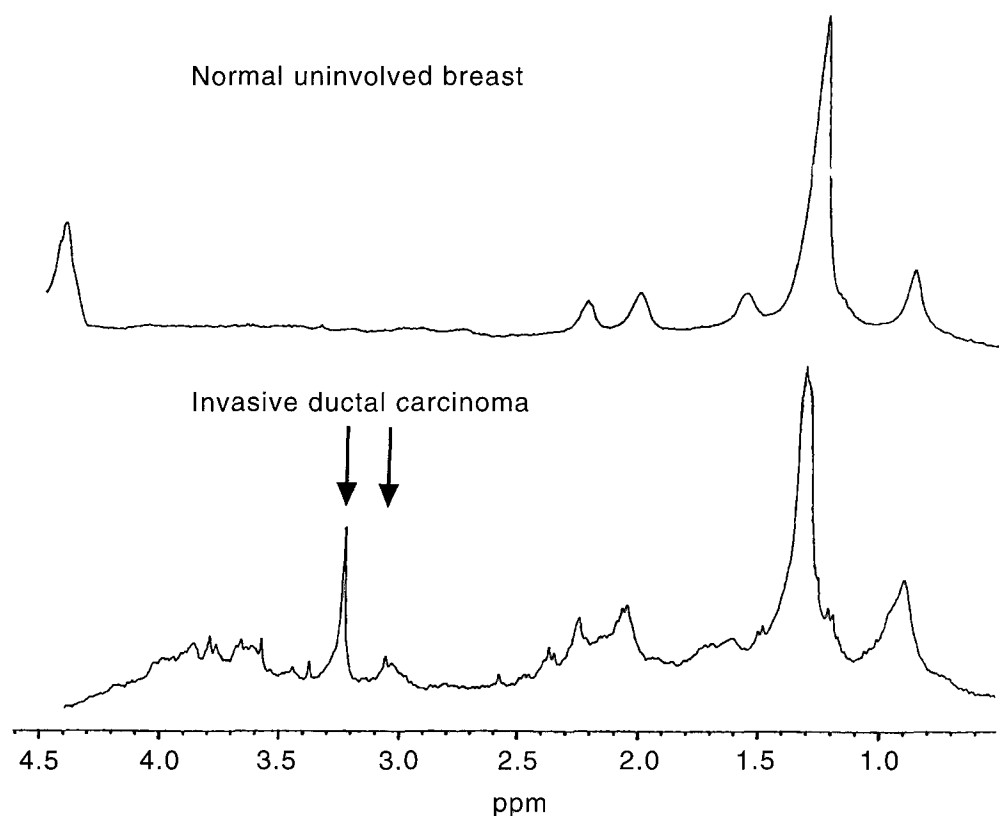


Fig. 2. Plot of the ratio of the intensity of resonances at 3.25 and 3.05 ppm, measured from magnetic resonance spectra as shown in Fig. 1. Unequivocally benign and invasive lesions are compared. Data are grouped on the basis of the final histopathology of tissue specimens taken from the aspiration site.

progressing from *in situ* to frankly invasive prior to morphological manifestation. Pure DCIS was not distinguished from benign lesions based on the ratios evaluated.

The breast study has been effective in further characterizing a group of patients where FNAB has been inconclusive or negative but where diagnosis remains unclear. The first application of MRS to breast disease may therefore be to reduce the false negative (sensitivity) biopsy rate by identifying suspicious lesions with malignant potential. Biopsy is currently necessary to prove that a lesion is benign.

Magnetic resonance spectroscopy on FNAB in breast lesions may therefore complement 'triple assessment' and reduce the need for unnecessary biopsy of benign lesions. This obviates the need for open biopsy prior to definitive therapy of invasive lesions by increasing diagnostic specificity of cytology. Pre-operative diagnosis of breast lesions by proton MRS offers benefits in patient management by reducing potential morbidity related to biopsy and allaying anxiety due to equivocal diagnosis.

LYMPH NODES

The first study undertaken to determine the sensitivity and specificity of MRS for detecting micrometastases was carried out on nodes from tumour-bearing rats.² In the present study, two-dimensional MRS, a more time-consuming method, was necessary due to the high levels of fat in the nodes. Micrometastases missed by conventional histological sectioning procedures were detected by MRS. Moreover, even when serial sectioning was used, MRS still identified micrometastases missed by the histopathologists. The presence of malignant cells was identified by the appearance of lactate, in the MR spectrum. At the disease stage when lactate but not choline was detectable by MRS, light microscopy failed to diagnose the presence of malignant cells. When MRS detected choline in addition to lactate, histopathology was also able to make the correct diagnosis. Thus, MRS could detect malignant cells prior to extensive proliferation, whereas the light microscope needed clusters of cells to make a diagnosis. The MRS method was supported by xenografting the nodes into nude mice.²

Recently, a collaborative programme between Dr Cynthia Lean from the IMRR and the NMR Centre at MGH, Harvard Medical School, has developed a new and faster method of collecting data on human nodes which will allow the detection of micrometastases in human nodal tissue in less than 12 min, examining the whole specimen (C.L. Lean *et al.*, unpubl. data).³

In yet another approach, assessment of FNA taken from nodes of breast cancer patients also offers significant promise as a routine method of biopsy and diagnosis by MRS.⁴

OTHER ORGANS

Follicular thyroid lesions

Magnetic resonance spectroscopy distinguishes benign and malignant subtypes of follicular thyroid lesions that cannot be discerned by light microscopy.² This method will now be evaluated within a clinical trial in which the 'benign' group will be offered conservative management and continued observation rather than invasive surgery.^{5,6}

Colon

The adenoma carcinoma sequence, first documented by Fearon and Vogelstein,⁷ was also able to be recorded by MRS. The identi-

fication, by MRS, of cell surface fucosylated molecules which increased in complexity with increased dysplasia was consistent with the adenoma carcinoma model.⁸

Prostate

Magnetic resonance spectroscopy subdivides histologically determined benign prostatic hypertrophy (BPH) into a genuinely benign form and one which is 'switched on' with some malignant features. This study also shows that determination of the correct pathology is best made when each of the four prostate zones are considered separately (P. Swindle, unpubl. data, 1998).⁹

Oesophagus

Barrett's oesophagus can be subdivided into a benign and a malignant subtype that cannot be determined by light microscopy.¹⁰

Confirmation by other sites around the world

The contentious nature of this programme has been overcome by sites overseas who verify the research. The National Research Council of Canada (NRC) has reproduced the IMRR data on ovary, colon, and cervix and was the first to publish *ex vivo* data on the prostate. The NRC's major contribution has been the development of multivariate analysis (artificial intelligence) to provide a robust objective data interpretation. This was first undertaken on the IMRR thyroid database.¹¹

CONCLUSION

Magnetic resonance spectroscopy, which is the original science behind MRI, has been 'rediscovered' to provide an objective method in determining whether a lesion is benign, in the malignant transfer process or truly malignant. When used as an adjunct with MRI it will offer a technology where lesions can be localized macroscopically by imaging and then diagnosed microscopically (at a cellular level) by spectroscopy: two technologies using the same principles and similar or the same equipment.

A second and most exciting aspect of the IMRR MR programme is the subcategorization of pre-invasive conditions such as DCIS of the breast, Barrett's oesophagus, and prostatic BPH where lesions already committed to malignancy but not detectable by light microscopy can be identified.

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BREAST CANCER AND MAGNETIC RESONANCE SPECTROSCOPY

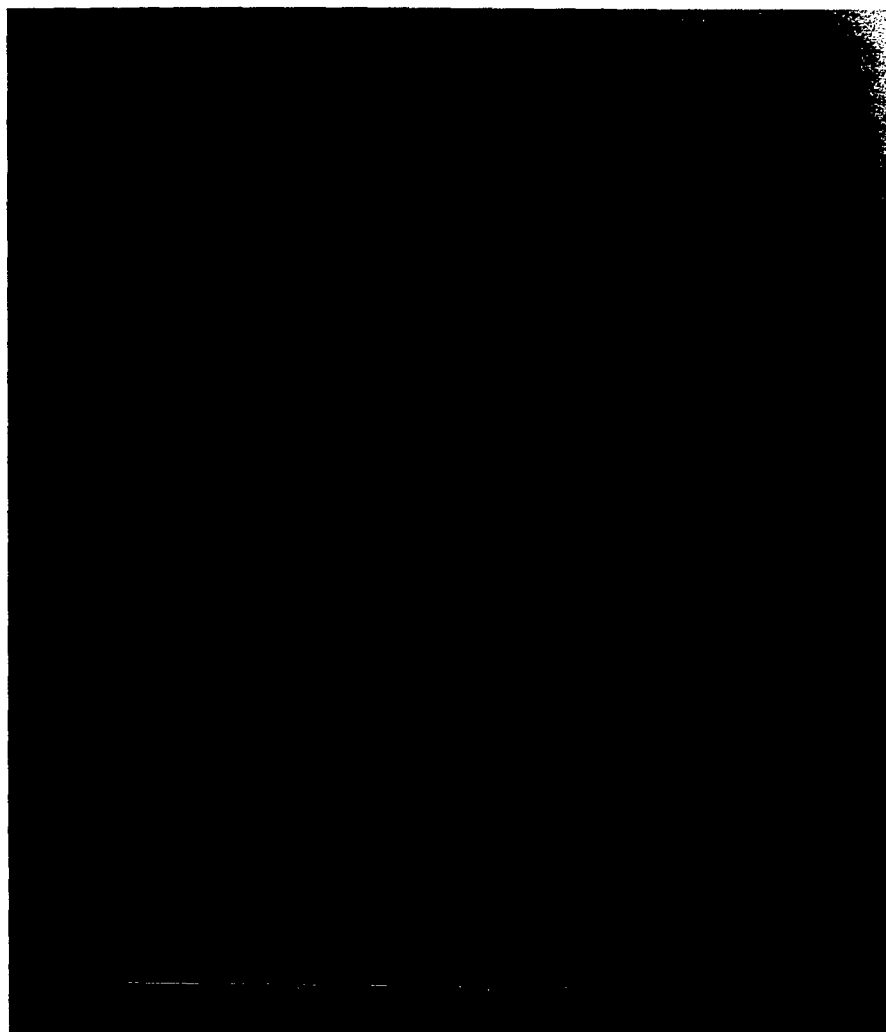
Proton magnetic resonance spectroscopy (MRS), which reports alterations to cellular chemistry, could monitor the development of breast tumours.

BY CAROLYN E MOUNTFORD

Earlier diagnosis and more effective management of breast cancer have led to improvement in breast cancer patient outcomes [1,2]. However combining physical examination, mammography and fine needle aspiration cytology (known as triple assessment) — the most sensitive way of diagnosing pre-operative clinical and radiographically detected breast lesions [3,4] — is flawed. Triple assessment has a high probability of detecting all malignant lesions but its sub-optimal specificity results in uncertainty, needing open biopsy (surgical intervention) to exclude malignancy.

Physical examination has limitations due to individual variation in breast consistency, the site and size of the lesion (less than 1cm is usually impalpable), and the presence of a diffuse versus discrete tumour. Screening mammography guidelines ensure that about one benign lesion is biopsied for every malignant lesion detected [3]. Even so, 10–40% of palpable breast cancers are missed by mammography alone, especially in women under 50 in whom radiographically dense breast tissue may mask changes associated with malignancy [4,5].

The golden standard for diagnosis, histopathology, relies on alterations to cellular morphology and tissue architecture. Fine needle aspiration (FNA) cytology has a sampling error of about 1–15% [6], partly explaining its variable sensitivity. While the complete sensitivity of FNA is 81–97% [7], this includes atypical and suspicious diagnoses that typically lead to histological confirmation of cancer by open biopsy.



The cornerstone of breast cancer diagnosis — triple assessment — might not be the only way of monitoring and diagnosing this disease. What part can magnetic resonance spectroscopy play?

sy in 50–80% of cases [8].

We have investigated the possibility that a new method, proton magnetic resonance spectroscopy (MRS) which reports on alterations to cellular chemistry, could monitor the development of breast tumours (Figure 1). MRS detects pools of chemicals that are mobile on the MR time scale. Therefore, the technology reports not on the total chemical content of the cells but on pools of chemicals that are active at a particular stage of development. By reporting such alterations to cellular chemistry that often do not manifest morphologically and therefore cannot be seen under the light microscope, MRS may provide a robust and accurate way of diagnosing breast cancer.

A technology like MRS, which monitors cellular chemistry correlating with different cell behaviour, could offer both independent and objective assessment of breast tissue. The potential then exists to identify a predisposition towards, or early features of, breast cancer and therefore offer interventions aimed at reducing tumour development. MRS is one diagnostic method that has been successfully applied to monitor tumour development and progression in other organs [7, 9–12].

Proton MRS can distinguish pre-invasive from invasive cervical cancer with a sensitivity and specificity of 98% and 94%, respectively [7]. It also distinguishes genuinely benign from malignant follicular lesions in human thyroid [12] and discriminates degrees of loss of cellular differentiation in ovarian tumours [11].

Preliminary investigations of excised breast tissue by 1-D and 2-D proton MRS detected increased levels of glycerophosphocholine and phosphocholine in invasive breast carcinoma compared with benign fibroadenomas [15]. Specimens of benign fibrocystic disease were characterised by an absence of resonances from choline, amino acids and other metabolites. However, we had problems obtaining adequate spectral resolution from excised breast due to the high adipose content of the tissue (Figure 2). The intense broad MR resonances from this fat often masked other diagnostic resonances in the 1-D spectrum. This problem is largely overcome by applying a T_2 filter (Carr-Purcell-Meiboom-Gill sequence) during data collection [13] and/or with post-acquisitional data processing [14].

A simpler remedy was to optimise specimen collection. Collection methods substantially affect the amounts of exogenous

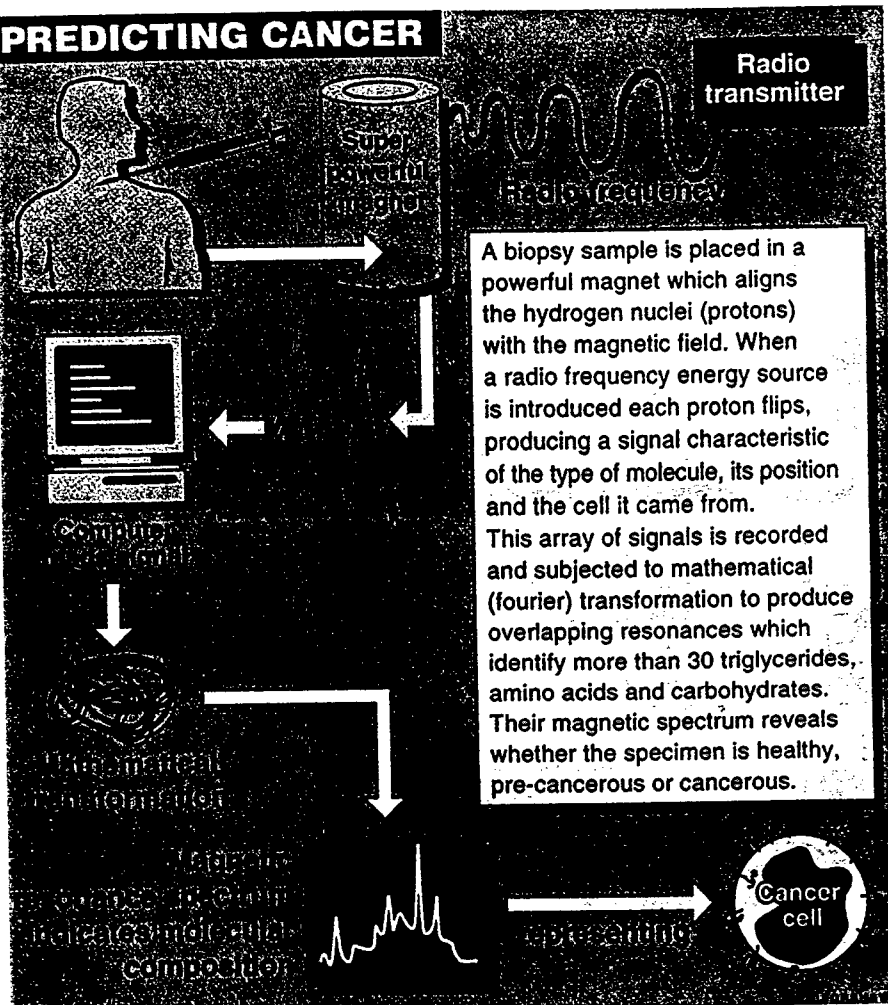


Figure 1. Predicting cancer with magnetic resonance spectroscopy (MRS).

fat sampled. FNA biopsy methods developed for thyroid sampling [12] provided cellular material adequate for assessment by MRS with reduced fat levels compared with excised tissue (Figure 2).

The study

Over 250 patients took part in the study [15], with a summary of histological diagnoses shown in Table 1. Fine needle aspiration biopsies (FNB) were taken from the breast and examined by MRS and then conventional histology. Comparisons were made to other pre-operative diagnostic investigations including physical examination, mammography and FNB cytology. Invasive cancer was identified by the presence of choline-containing metabolites. MRS discriminated between invasive cancer and normal or benign tissue based on the amount of choline when standardised to the creatine, phosphocreatine and lysine present in the same sample. MRS on FNB,

when compared with the histopathological diagnosis, has a sensitivity and specificity for distinguishing invasive carcinoma from benign breast lesions of 95% and 96% respectively, based on the 1-D peak intensity ratio of 3.25/3.05ppm (Figure 3 and Table 2). This data has now been sent to the National Research Council of Canada for a team of mathematicians to produce an algorithm to allow a robust, non subjective diagnosis independent of human involvement [14]. Preliminary outcomes place the sensitivity and specificity close to 99% [1–6].

All samples reported as ductal carcinoma-in situ (DCIS) by routine hospital histopathology ($n = 21$), were grouped according to the correlative histopathology on the MR specimen. MRS subcategorised DCIS into two categories. In category A (high choline to creatine/lysine ratio) were four DCIS samples where ductal cells had breached the basement mem-



Figure 2. MRS on human breast (ex vivo). 8.5 Tesla MRS spectra obtained from (a) breast tissue from a histologically diagnosed invasive tumour; (b) same tissue as (a) but dissociated mechanically; and (c) FNA biopsy from a patient with invasive breast cancer.

Benign	
Fibrosing changes	68
Fibroadenoma	15
Ductal hyperplasia (mild/florid)	9
Fat necrosis	4
Secretory adenosis	2
Radial scar	2
Atypical ductal hyperplasia	2
Duct ectasia	2
Miscellaneous	2
Total	106
Phyllodes tumour	2
Ductal carcinoma in-situ	
DCIS (high grade)	10
DCIS (low grade)	1
DCIS (comedo necrosis)	2
DCIS + microinvasion	4
Total	17
Invasive carcinoma	
Ductal carcinoma (NST)	66
Ductal carcinoma (NST), EIC positive	7
Lobular carcinoma	5
Tubular carcinoma	4
Total	82

Table 1. Summary of histological subtypes of FNB samples. DCIS = Ductal carcinoma in situ; NST = no special type; EIC = extensive intracutaneous component. Histopathology according to dominant findings on correlative histopathology samples.

brane (under 1mm) in one or more focus and microinvasion was identified in another four samples. This group of eight as well as two samples of high grade DCIS with extensive comedo necrosis were malignant by MR criteria. Group B containing DCIS (10 high grade, 1 low grade) all had a low choline to creatine/lysine ratio similar to that obtained for benign lesions.

Therefore, MRS on FNB clearly distinguished pure DCIS without comedo necrosis or microinvasion from invasive carcinoma. However, even when DCIS specimens contained comedo necrosis or a few foci of

microinvasion, MRS ranked the specimen in the invasive category in every case. This may indicate chemical changes occurring in cells progressing from in situ to frankly invasive before morphological manifestation. Pure DCIS was not distinguished from benign lesions based on the 1-D 3.25/3.05ppm ratio.

MRS on FNB may therefore complement triple assessment and reduce the need for unnecessary biopsy of benign lesions, while obviating the need for open biopsy before definitive therapy of invasive lesions by increasing diagnostic specificity of cytology. Pre-operative diagnosis of breast lesions by proton MRS therefore offers potential benefits in patient management by reducing potential morbidity related to biopsy and allaying anxiety due to equivocal diagnosis.

However, a most exciting aspect of this program is the subcategorisation of DCIS where some lesions already are committed to malignancy but are not detectable by light microscopy.

Acknowledgements

The multi-disciplinary nature of this project involved surgeons, pathologists, scientists and technologists from the Institute for Magnetic Resonance Research based at the University of Sydney. These include Bruce Barraclough, Peter Barry, Michael Bilous, Sinead Doran, Susan Dowd, David Gillett, Cynthia Lean, Wanda

BREAST FNB Benign vs Infiltrating Carcinoma

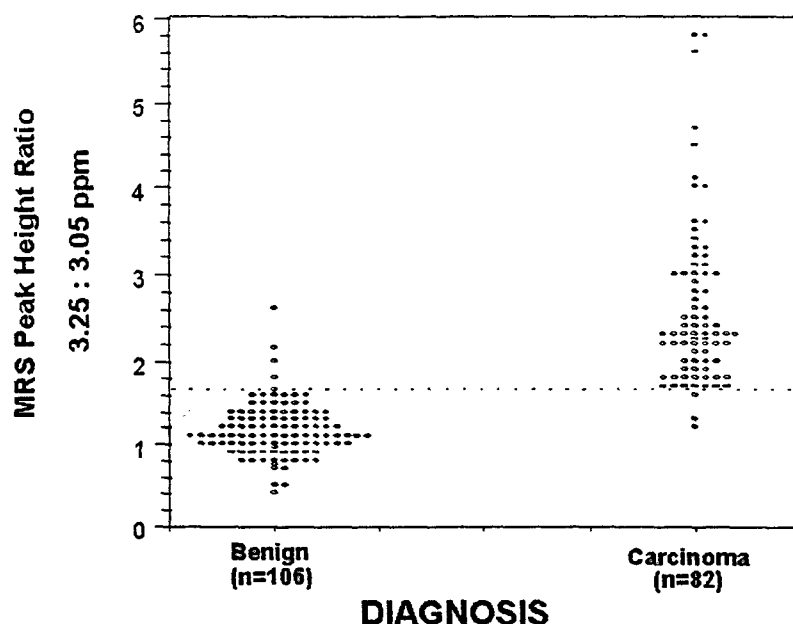


Figure 3. Plot of ratio of intensity of resonances at 3.25 and 3.05ppm measured from MR spectra as shown in Figure 2. Benign and invasive lesions (unequivocal) are compared. Data are grouped on the basis of the final histopathology of tissue specimens taken from the aspiration site.

	Invasive carcinoma	Normal/benign	
MRS (FNB) positive	78	4	PPV 95%
Ratio ≥ 1.7	(TP)	(FP)	(78/82)
MRS (FNB) negative	4	102	NPV 96%
Ratio under 1.7	(FN)	(TN)	(102/106)
	Sensitivity 95%	Specificity 96%	Total = 188
	(78/82)	(102/106)	

Table 2. Test result probabilities for diagnosis by MRS on FNB compared with histological diagnosis on tissue. TP = true positive; FN = false negative; FP = false positive; TN = true negative; PPV = positive predictive value; NPV = negative predictive value; Sensitivity or true-positive rate = frequency of positive test result (ie. MRS ratio 1.7) in those with malignant disease; Specificity or true-negative rate = frequency of negative test result (MRS ratio under 1.7) in those without malignant disease as judged by histopathology of breast tissue excised from the aspiration site.

Mackinnon and Peter Russell.

Carolyn Mountford is executive director of the Institute of Magnetic Resonance Research at the University of Sydney.

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Next month in

today's LIFE SCIENCE

In June, we bring you the second of our quarterly microbiology supplements, *Today's Microbiology*.

This month's focus is Environmental Microbiology, which covers:

- microbial indicators of river health
- stunning images from the Image of the Year award
- book reviews
- microbiology products

Next month, we also:

- preview the Haematology Society of Australia/Australian Society for Blood Transfusion conference
- focus on immunoassay/serology products
- publish the LabLink Catalogue

Out at the end of June

FNA stored at -70°C less than 6 months

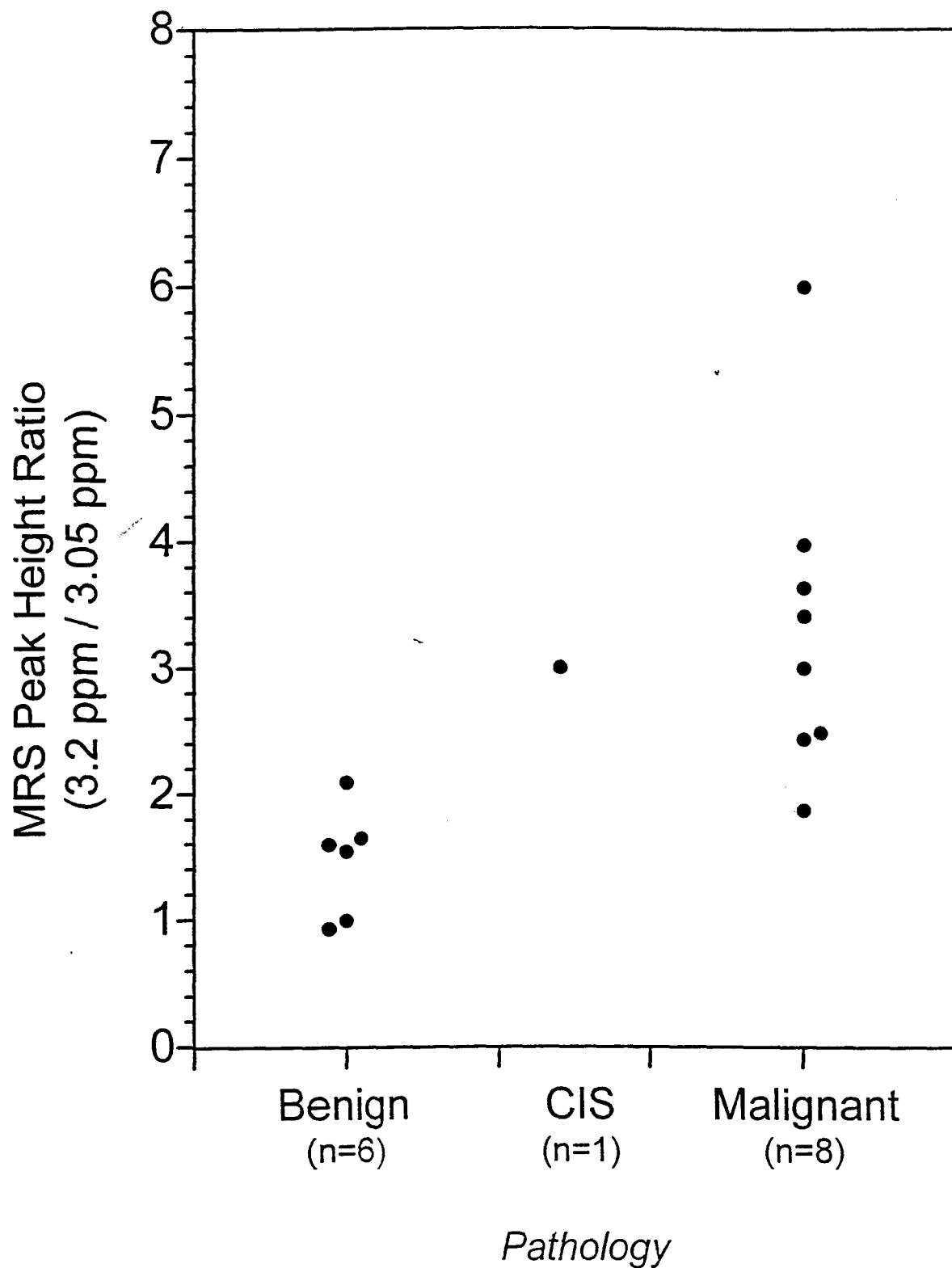


Figure 1A: Breast FNA specimens: MR ratios of benign, carcinoma and carcinoma *in situ* specimens stored less than 6 months at -70°C.

FNA stored at -70°C for 6-22 months

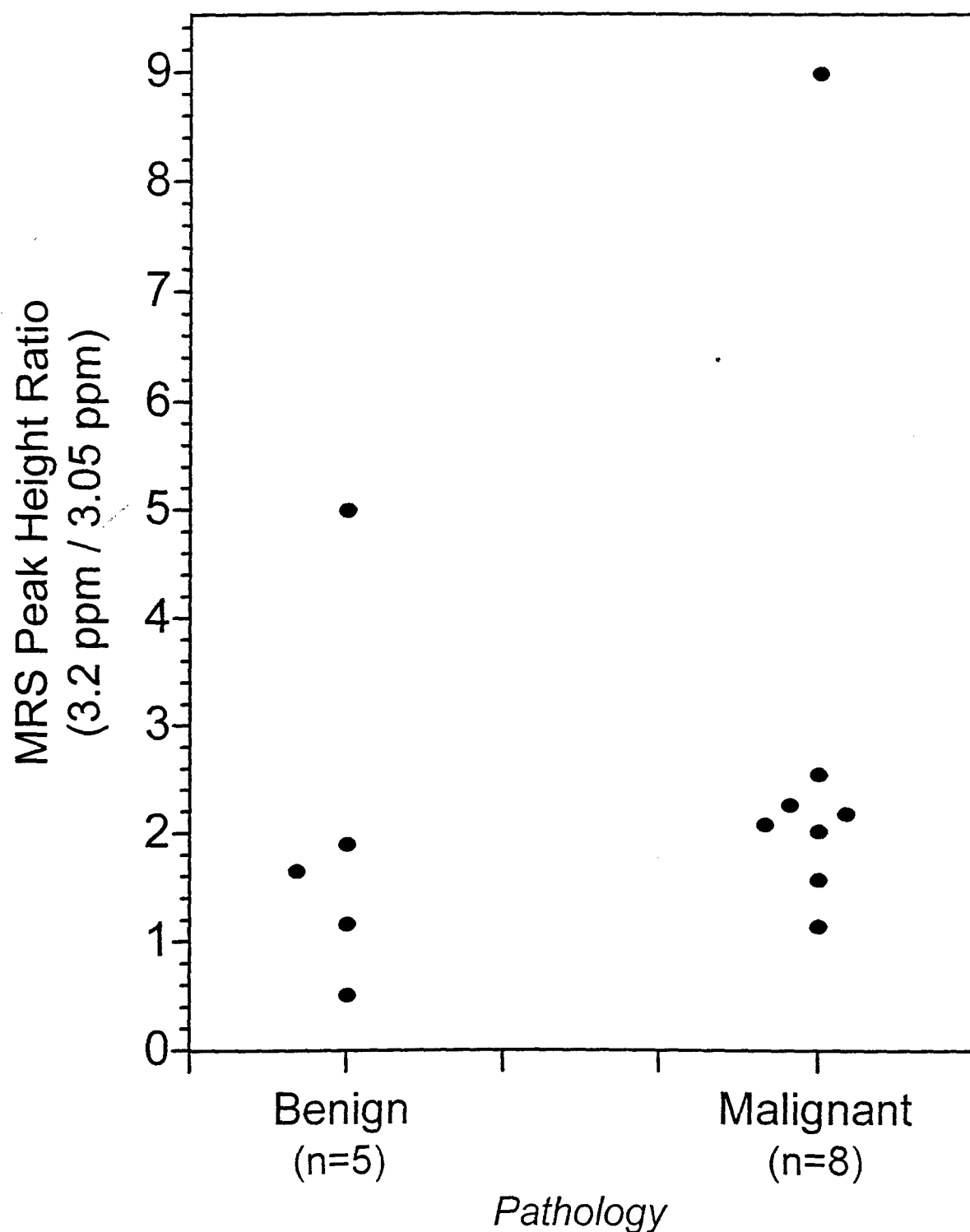


Figure 1B: Breast FNA: MR ratios of benign and carcinoma specimens stored longer than 6 months and less than 22 months at -70°C.

FNA specimen collected from breast
lesion *in vivo*

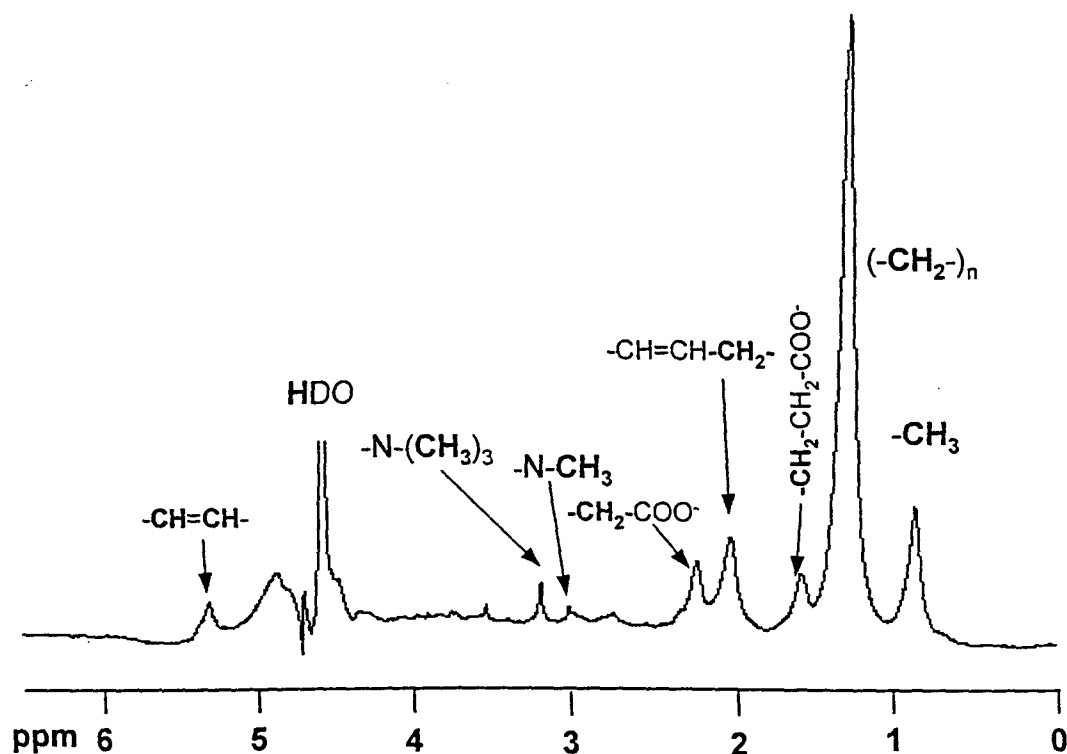
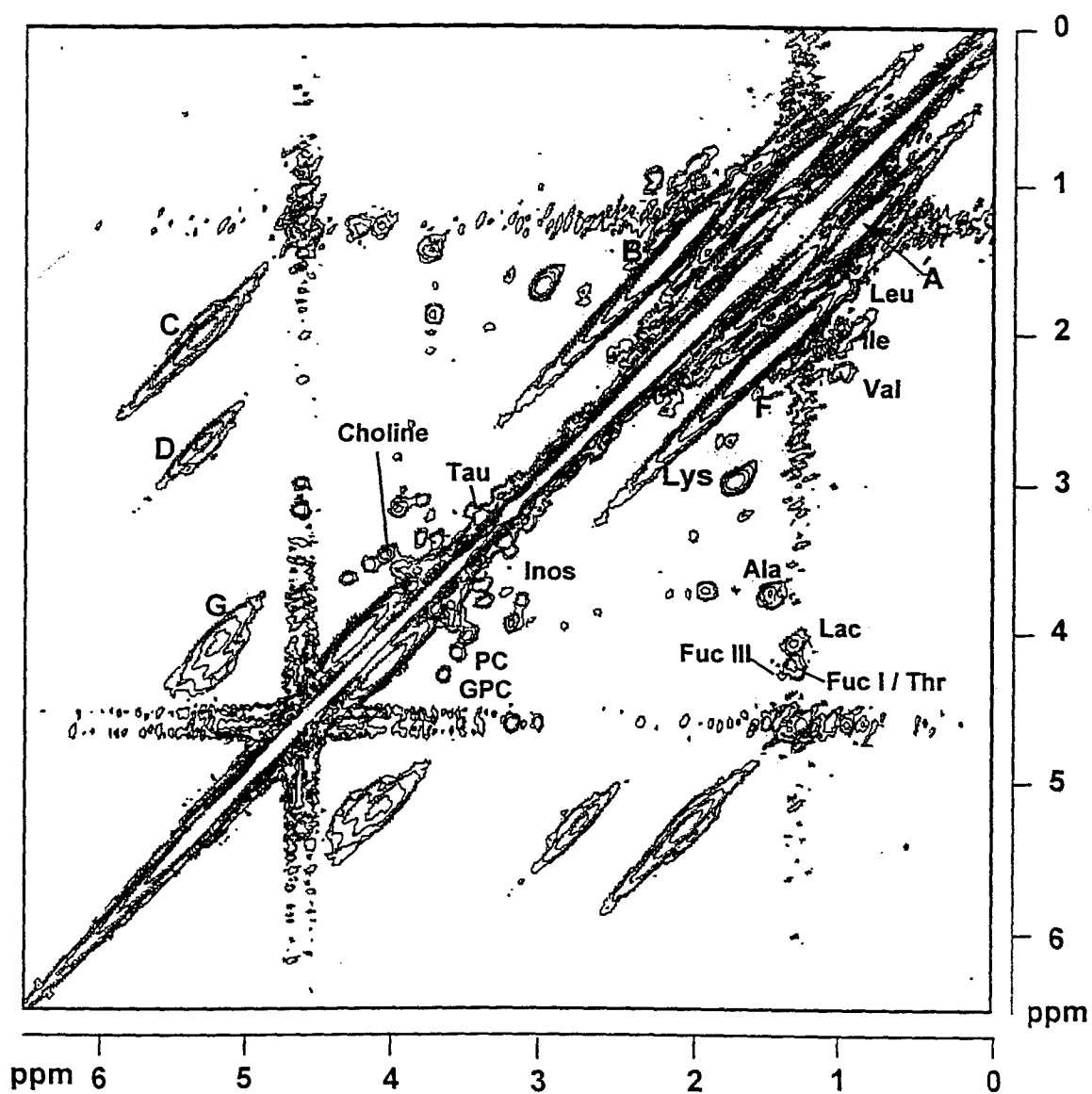
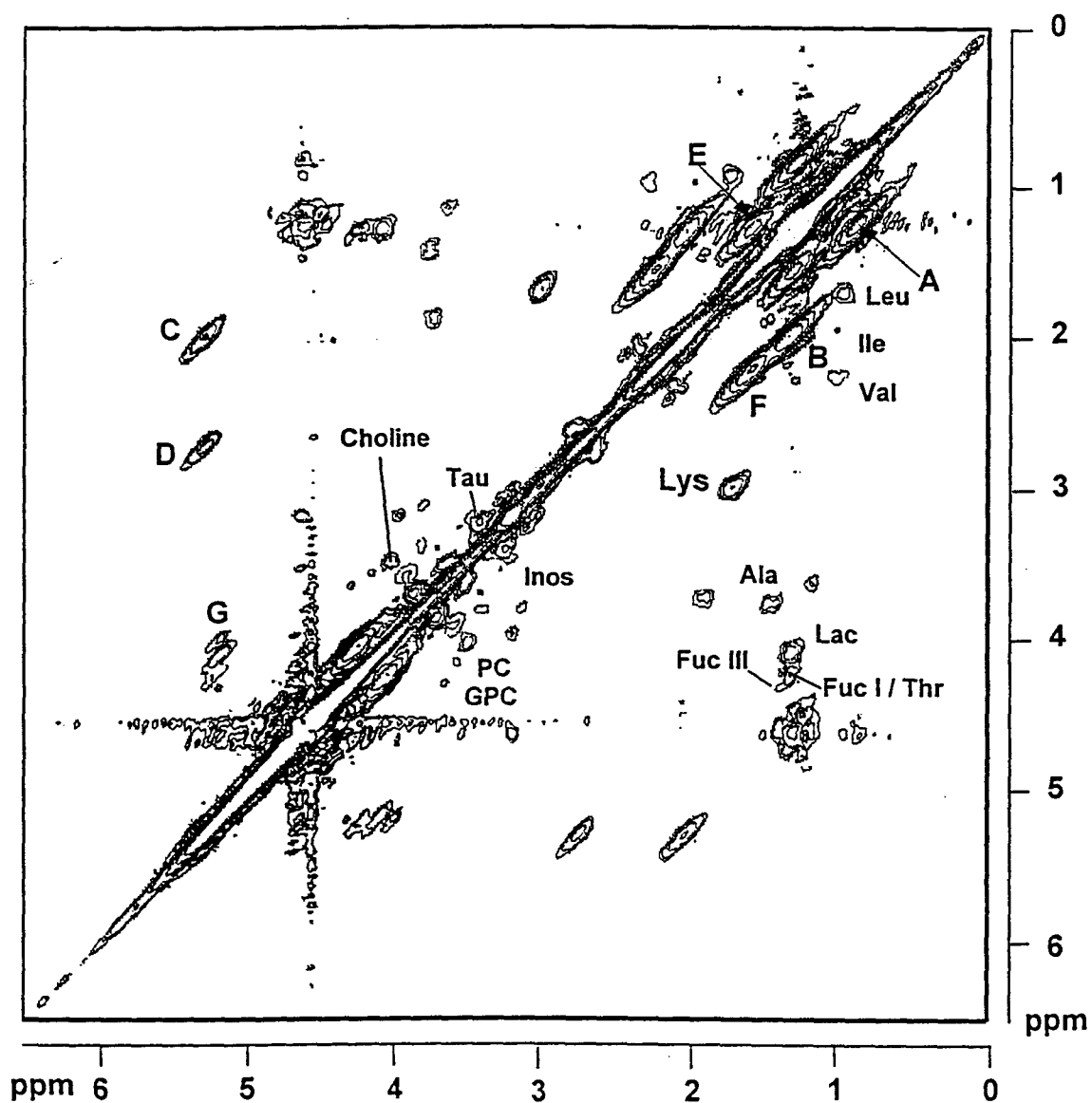


Figure 2: ^1H MR 1D spectrum (360 MHz) of malignant breast FNA collected *in vivo*. Both specimens have malignant histology. Spectra (256 scans, sweep width 3597 Hz) were collected at 37°C with the sample spinning at 20 Hz on a Bruker AM360 MR spectrometer. Residual water was suppressed by selective gated irradiation. A line broadening of 3 Hz was applied before Fourier transformation.

3A: Malignant (tissue specimen)



3B: *Carcinoma in situ* (tissue specimen)



3C: Benign (tissue specimen)

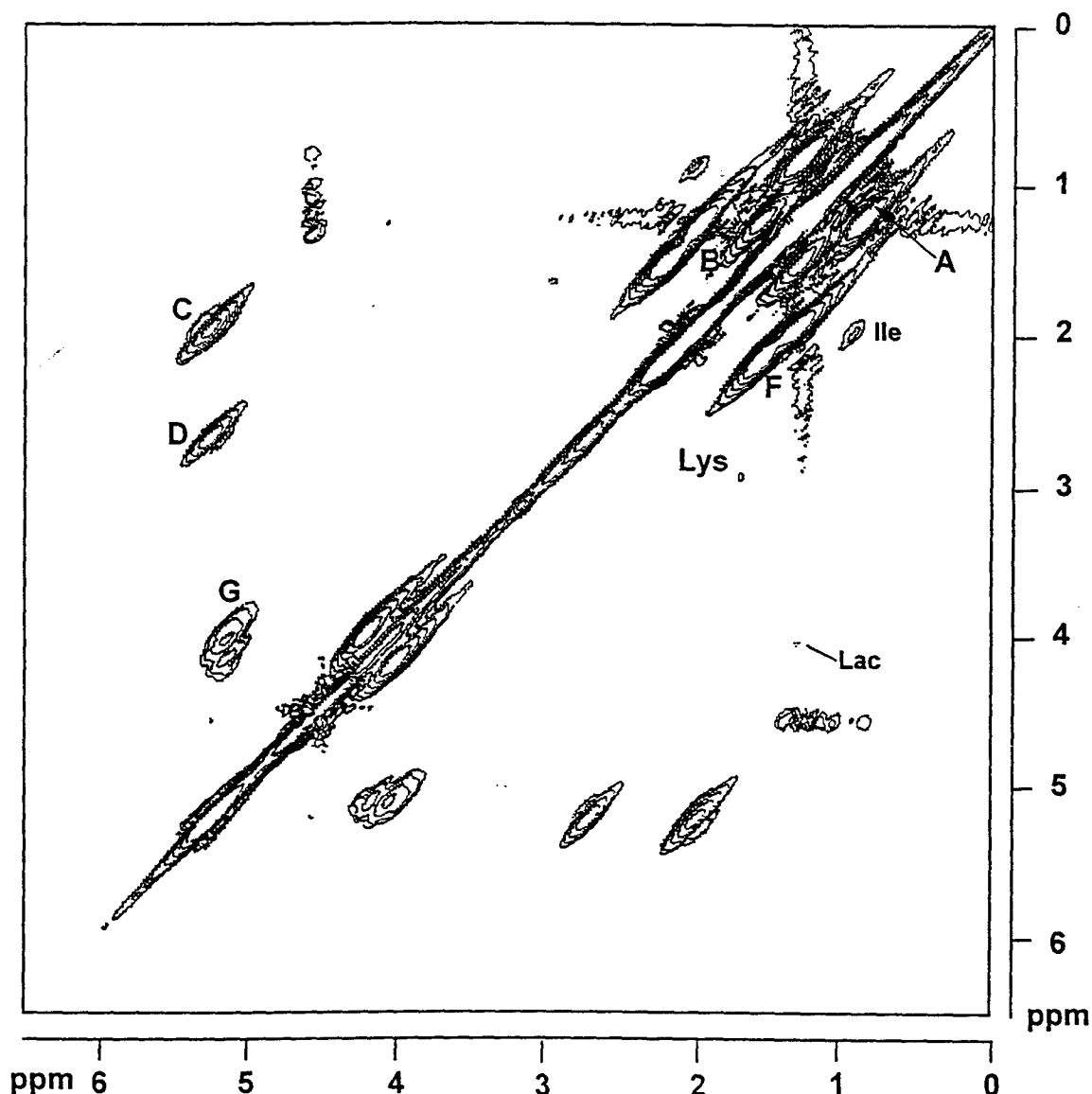


Figure 3: 360 MHz ^1H MR symmetrized COSY spectra of breast tissue specimens in $\text{PBS}/\text{D}_2\text{O}$, (NS=32, NE=200). A) Malignant; B) *Carcinoma in situ*; C) Benign tissue. Data were collected at 37°C with sweep width 3597 Hz. A sinebell window function in t_1 domain, and a Lorentzian-Gaussian window function (LB = -40, GB = 0.15) in the t_2 domain were applied before Fourier transformation.

Specimen collected intraoperatively

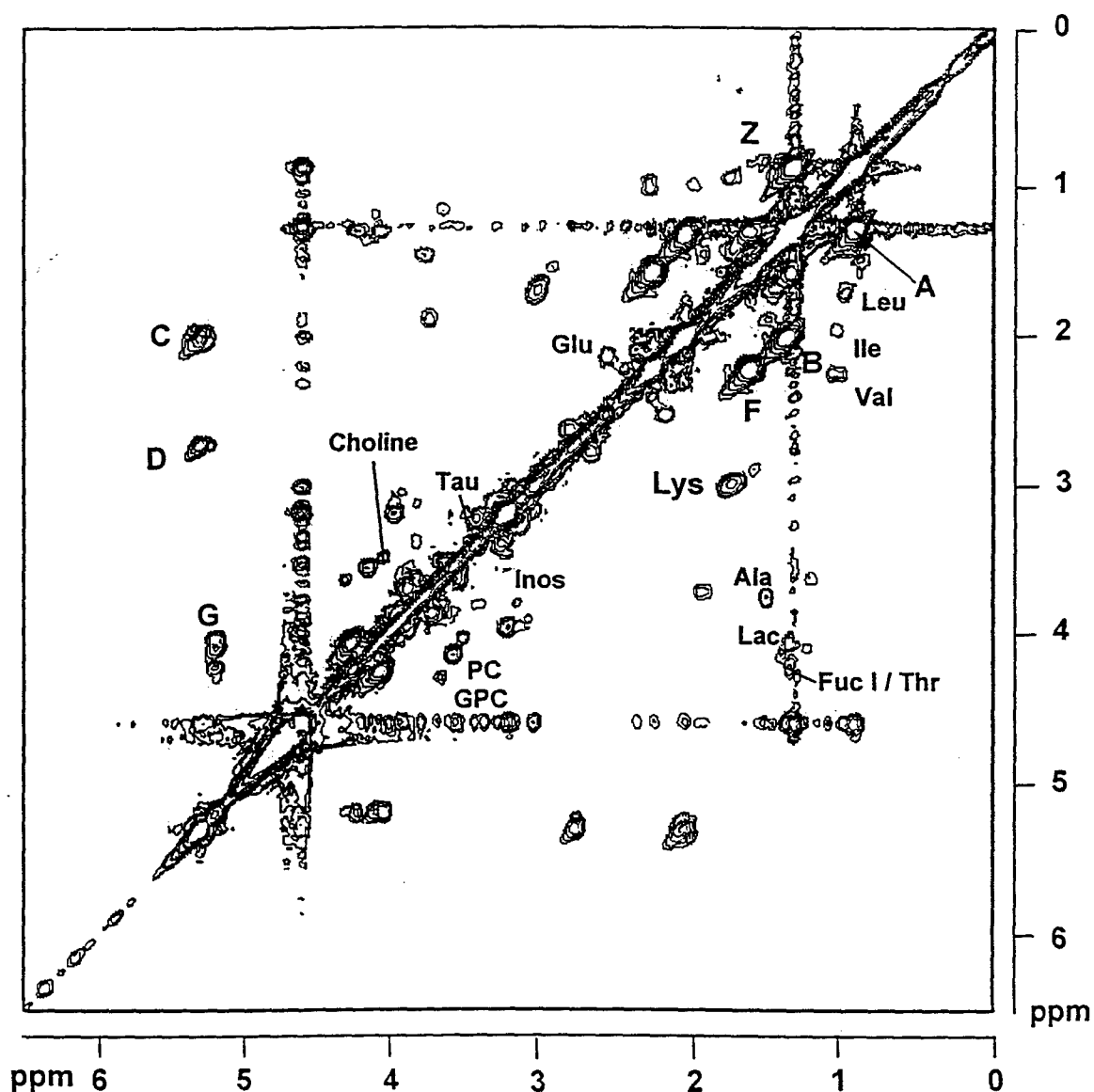


Figure 4: 360 MHz ^1H MR symmetrized COSY spectra of malignant breast FNA specimen, (NS=32, NE=200). Data were collected at 37°C with sweep width 3597 Hz. A sinebell window function in t_1 domain, and a Lorentzian-Gaussian window function (LB = -40, GB = 0.15) in the t_2 domain were applied before Fourier transformation.

Figure 5

Methyl-methine coupling region from COSY

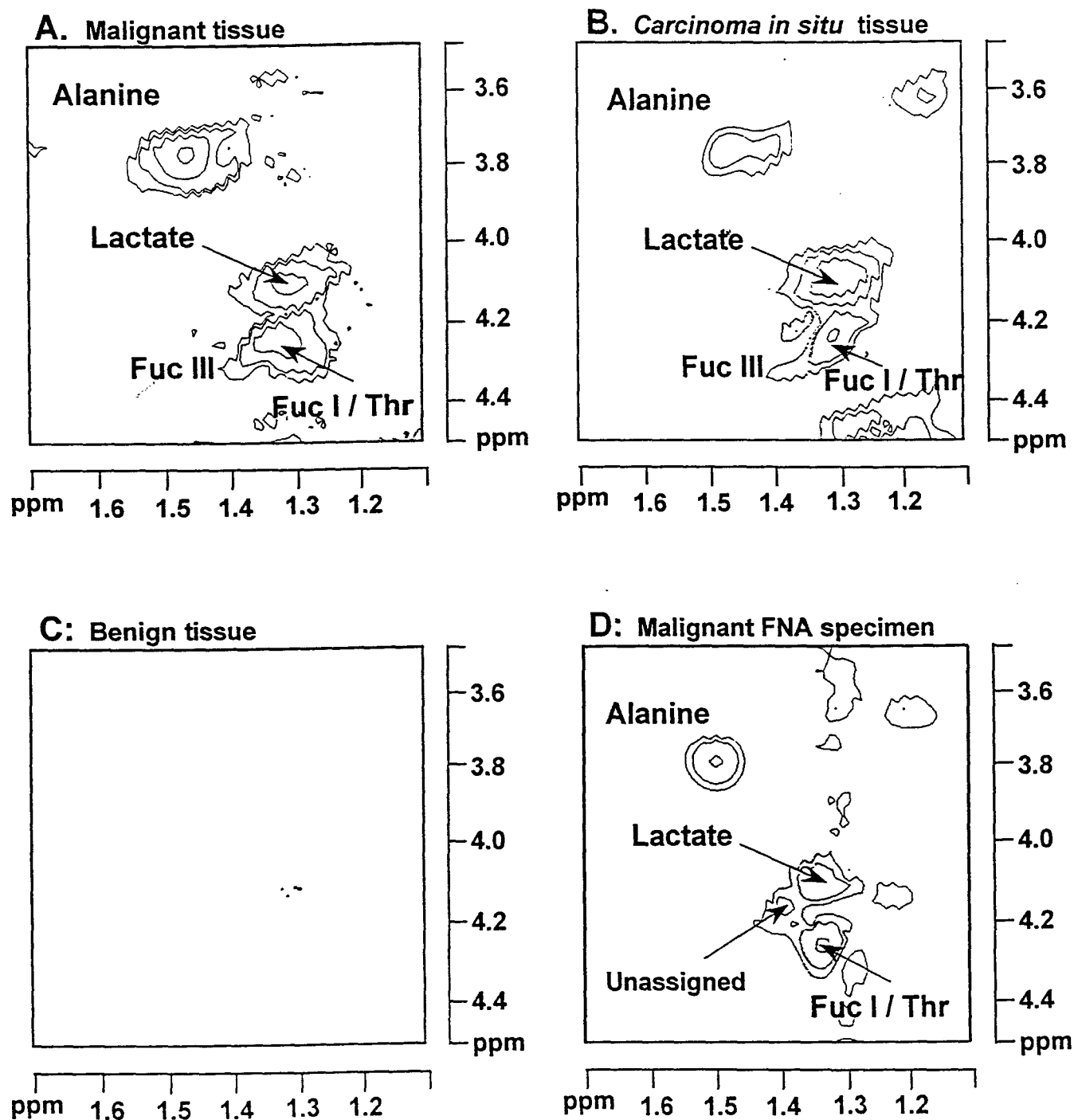


Figure 5: methyl - methine coupling region ($F_1 = 3.5 - 4.5$ ppm, $F_2 = 1.1 - 1.7$ ppm) from 360 MHz ^1H MR symmetrized COSY spectra of breast specimens (NS=32, NE=200). A) malignant tissue; B) *Carcinoma in situ* tissue; C) benign tissue; D) malignant FNA. Data were collected and processed as described in Figure legends 3+4.

Human Lymph Node Tissue Assessed by MRS

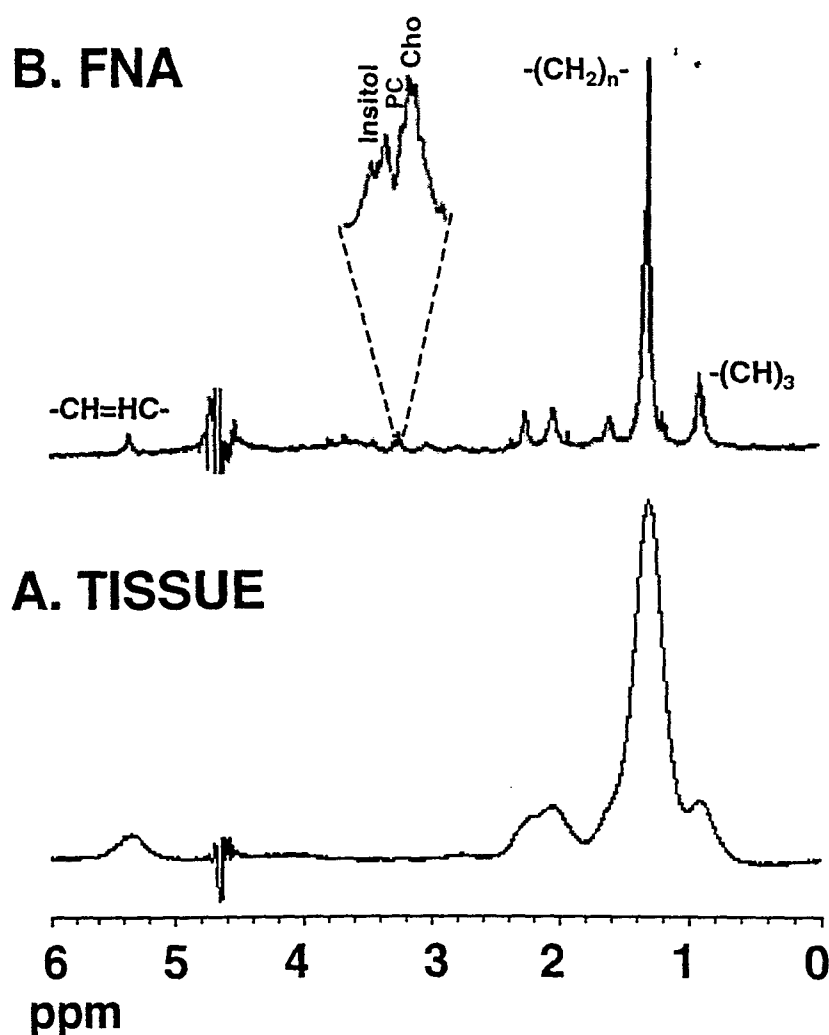
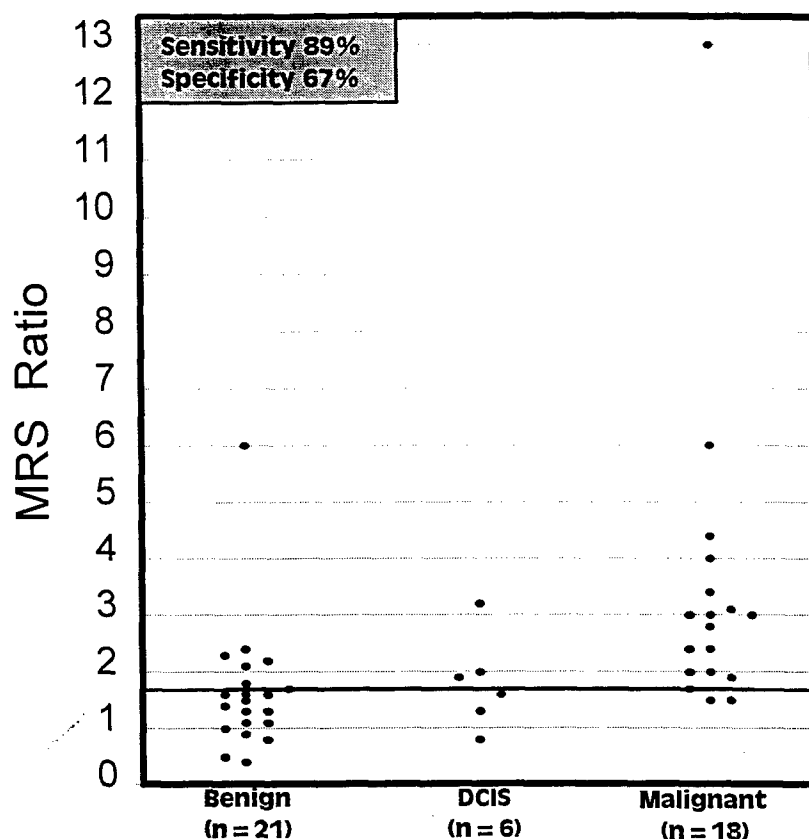


Figure 6: ^1H MR spectra (360MHz) of human lymph nodes. A. Tissue B. FNA biopsy. Spectra (128 scans, sweep width 3597 Hz) were collected at 37°C with the sample spinning at 20 Hz on a Bruker AM360 MR spectrometer. Residual water was suppressed by selective gated irradiation. A line broadening of 1Hz was applied before Fourier transformation.

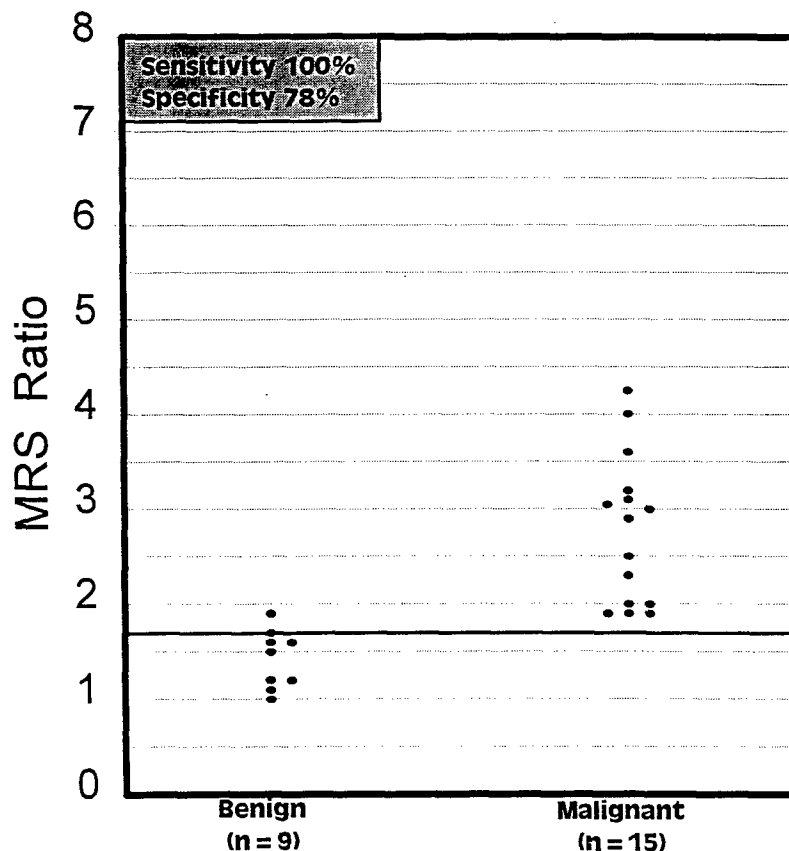
Data Analysed by Visual Inspection

A



Standard MRS
Protocol
(n=45)

B



Automated
MRS Protocol
(n=24)

Figure 7. MRS data collected from FNA of breast lesions during 1998/99.

A - MRS FNA data collected using standard MRS protocol;

B - MRS FNA data collected using newly developed automated MRS protocol outlined in Figure 8.

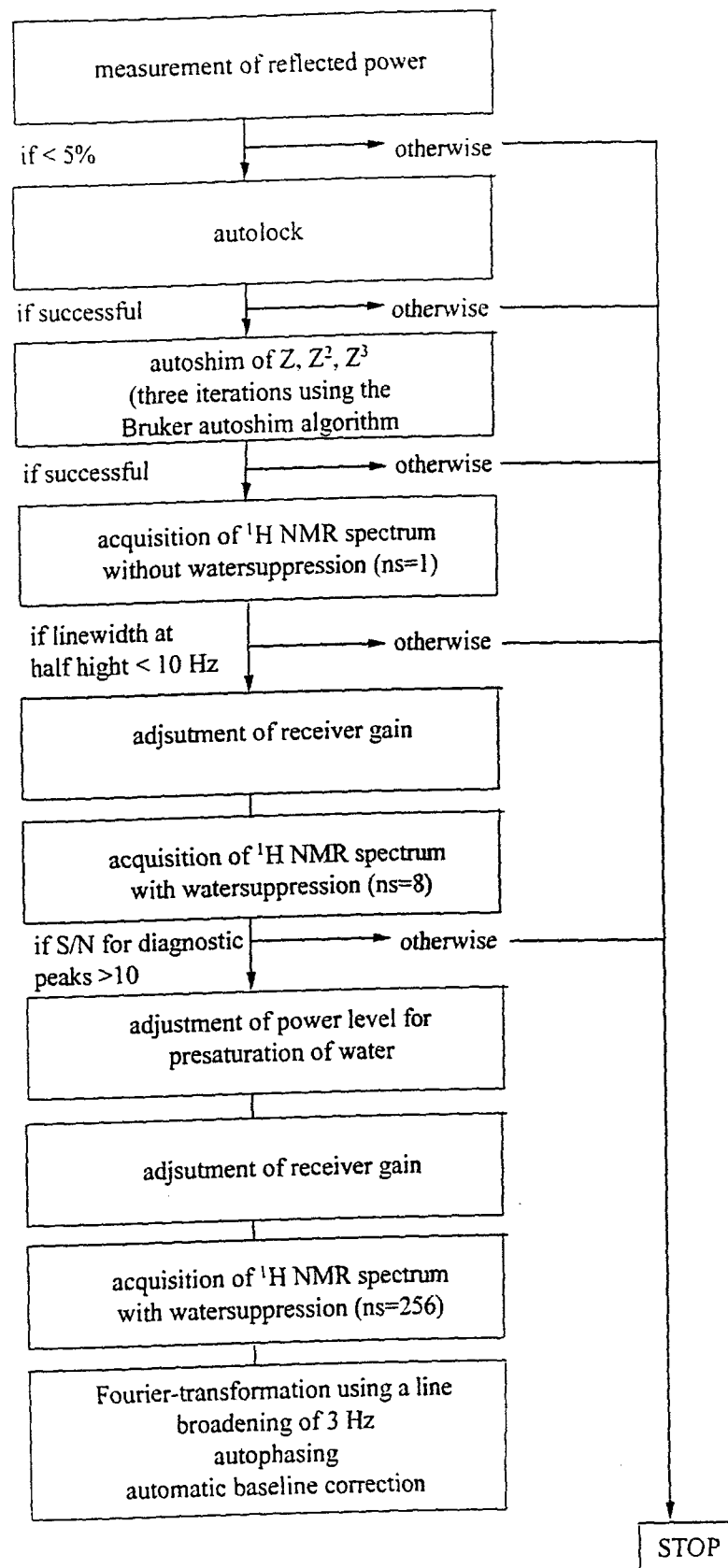
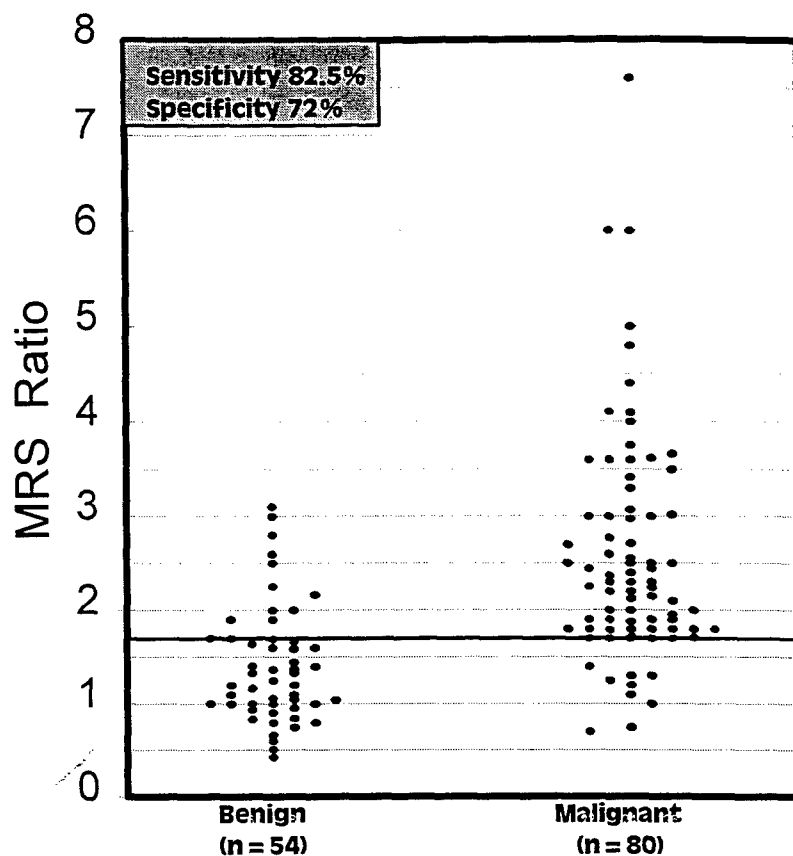


Figure 8: Simplified algorithm for automatic data acquisition and processing of ^1H MR spectra of breast FNB

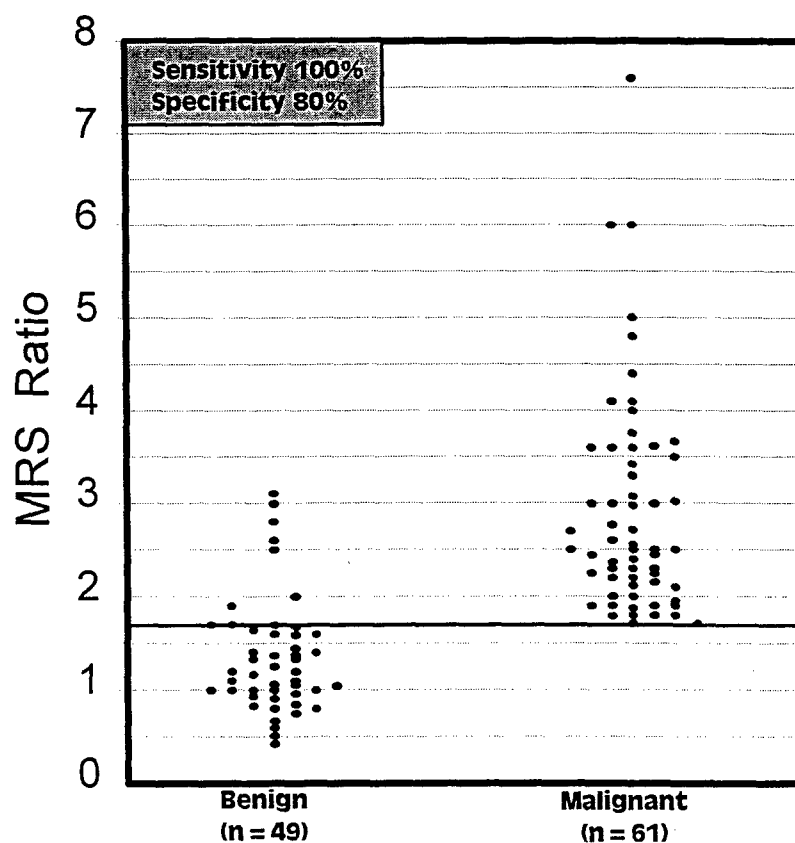
Data Analysed by Visual Inspection

A



All Data Suitable For
CCD analysis
(i.e. spectra with adequate
H₂O suppression and no
contaminants)
(n=134)

B



Data Suitable For CCD
Analysis With SNR_≥25
(n=110)

Figure 9. MRS data collected from FNA of breast lesions. The MRS ratio was calculated following visual inspection of the spectra and determination of peak height ratios. This figure shows the MRS ratio correlated with the histopathology of the lesion.
A - All MRS FNA data suitable for multivariate CCD analysis
B - MRS FNA data suitable for multivariate CCD analysis with SNR_≥25

Improved Water Suppression Method

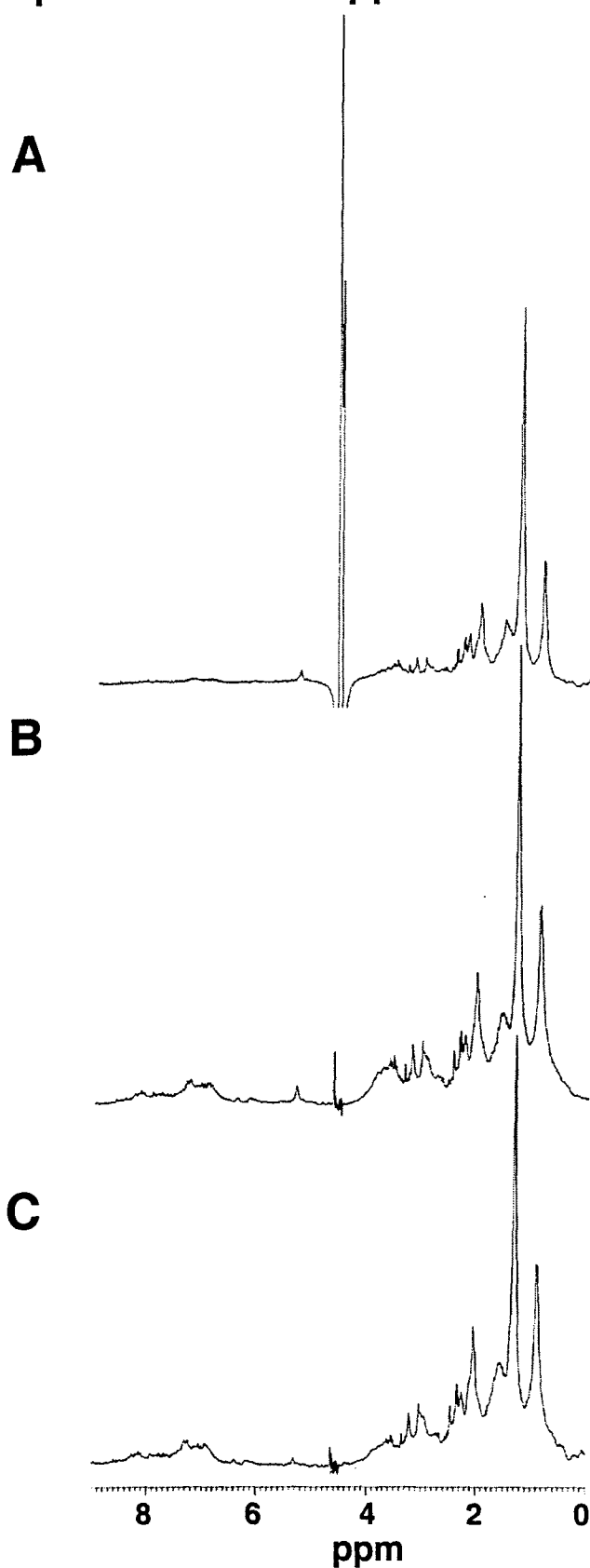


Figure 10: Spectra obtained from the same breast FNA at 37°C. A) Spectrum obtained with presaturation for 2s (water suppression ~600). B) Spectrum obtained with a double PFG echo sequence with a [soft 180°_x hard 180°_x] refocusing element, $\tau=500\mu\text{s}$ (water suppression ~15000). C) Same as B) with $\tau=600\mu\text{s}$ (water suppression ~17000). The absolute intensity scale is the same in all spectra.

MRS on FNA of Human Lymph Nodes

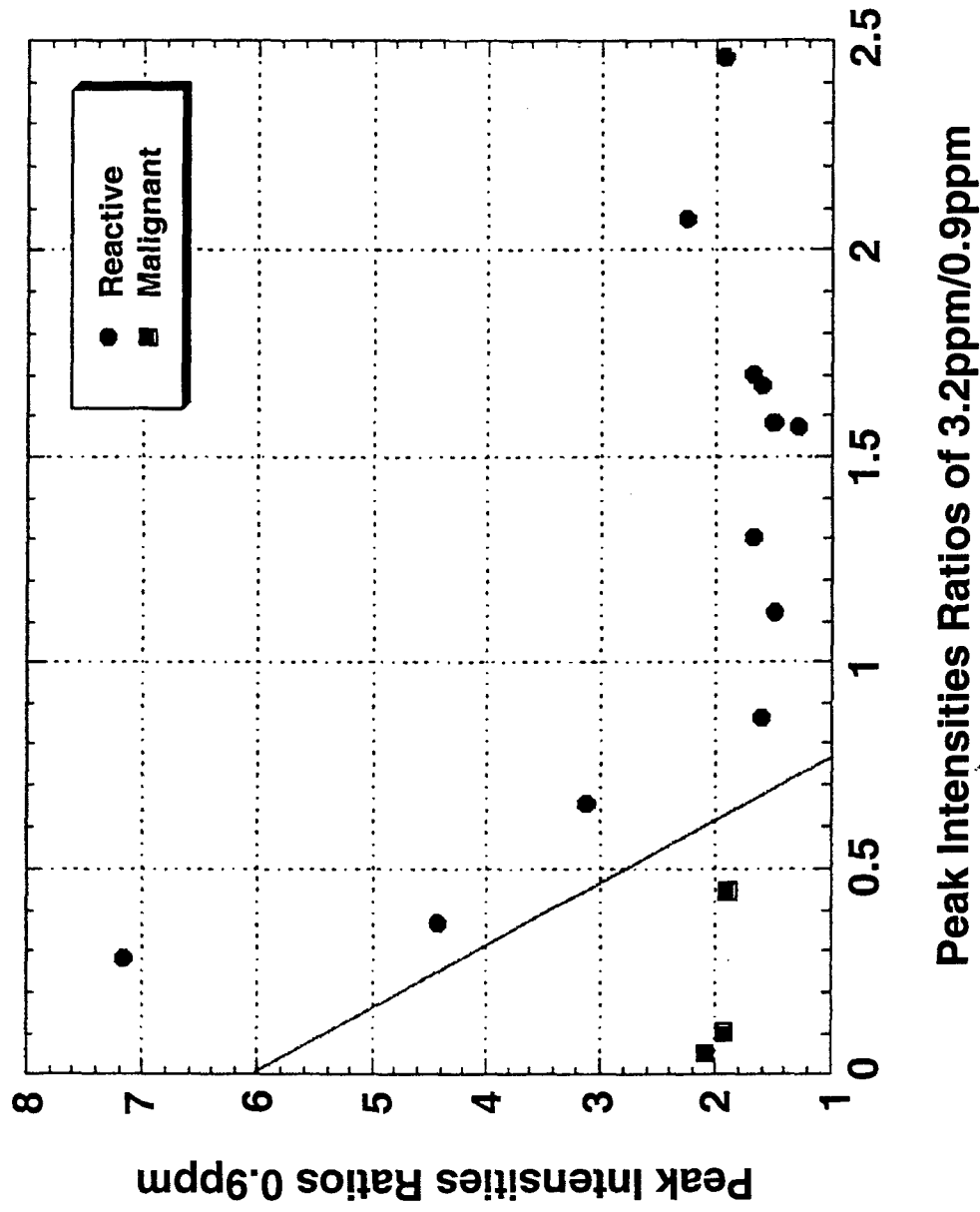


Figure 11 A 2-Dimensional Plot of MRS Data from FNAs of Malignant and Reactive Lymph Nodes. The Data were Derived from Spectra Resolution Enhanced using a Gaussian Lorentzian Transformation (GB=0.08, LB=-7.0)

Figure 12: ^1H MAS MRS of Human Lymph Nodes

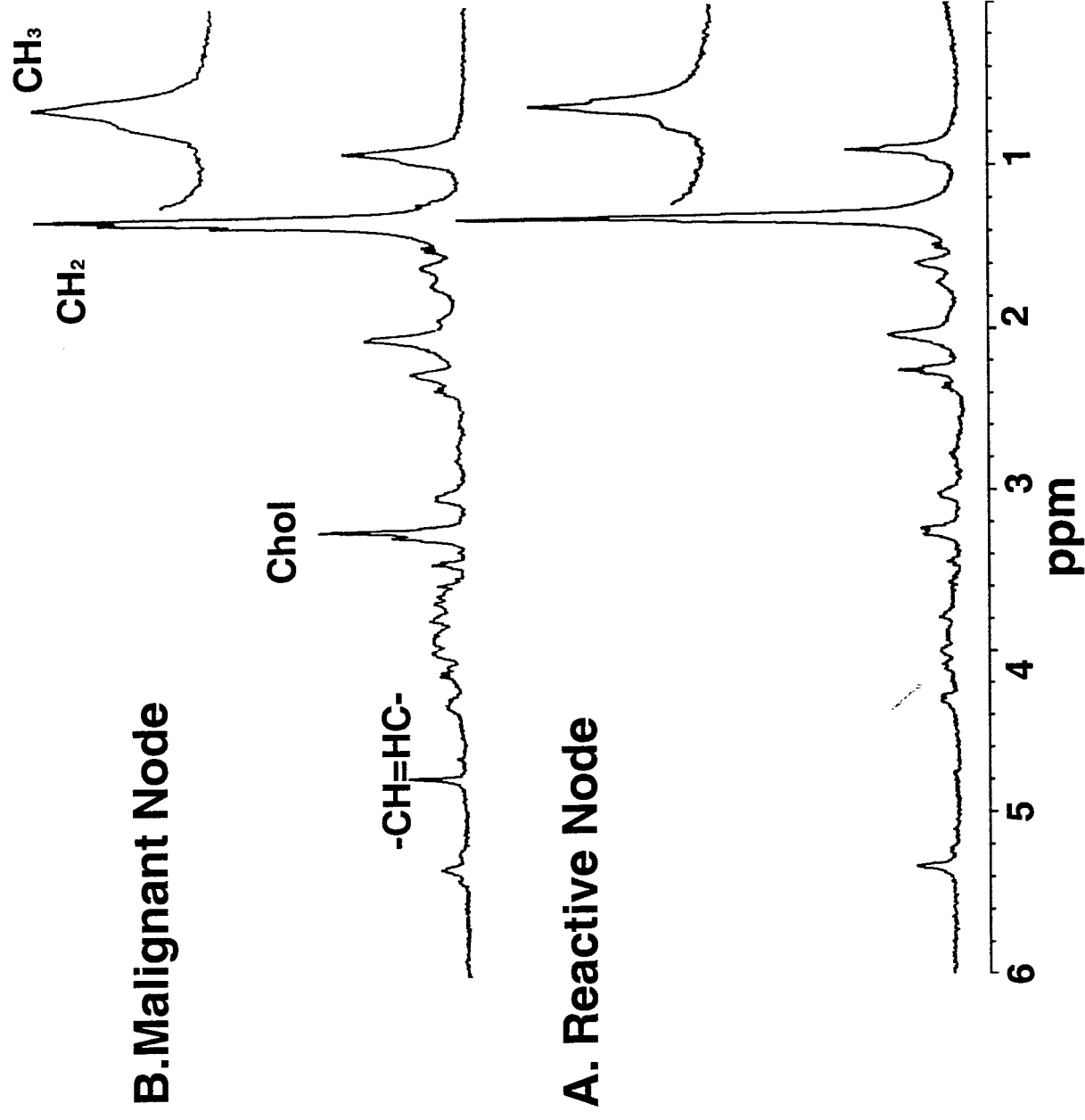


Figure 13: ^1H MAS MRS of Human Axillary Lymph Nodes

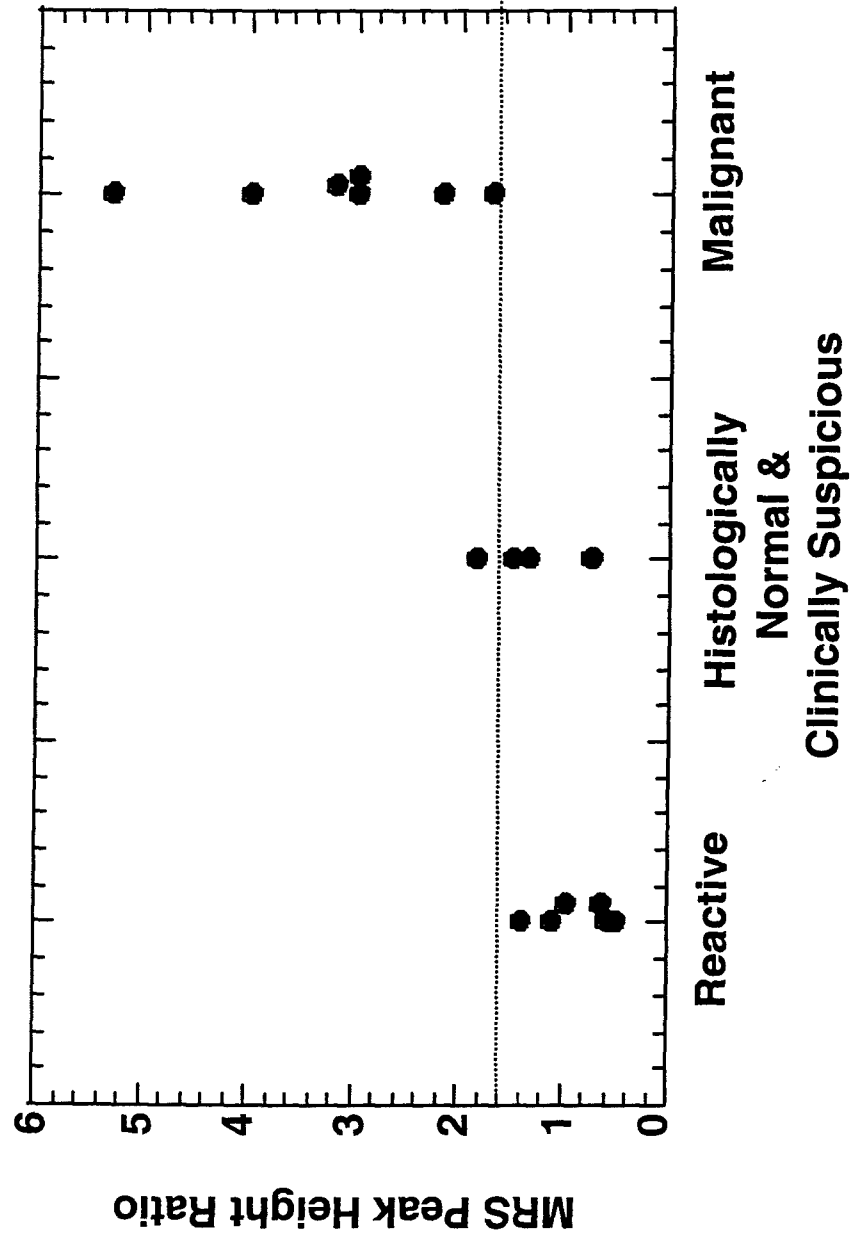


Table 1. Chemical species identified in 2D ^1H MR spectra of human breast specimens.

Molecules	Abbreviation	Coupling Partners (bold face)	Cross-peak Coordinates (ppm)
LIPIDS			f 2, f 1
Cholesterol	Z	$\text{CH}_3\text{-CH-ring}\alpha$	0.85, 1.52
Choline	Chol	$(\text{CH}_3)_3\text{N}^+\text{-CH}_2\text{-CH}_2\text{-OH}$	3.50, 4.07
Glycero-PC	GPC	$(\text{CH}_3)_3\text{N}^+\text{-CH}_2\text{-CH}_2\text{-OP(O)-O-CH}_2\text{-}$	3.69, 4.38
Phosphoethanolamine	PE	$(\text{CH}_3)_3\text{N}^+\text{-CH}_2\text{-CH}_2\text{-OPO}_3^-$	3.30, 4.14
Phosphocholine	PC	$(\text{CH}_3)_3\text{N}^+\text{-CH}_2\text{-CH}_2\text{-OPO}_3^-$	3.61, 4.25
Triacylglycerol	A	$\text{-CH}_2\text{-CH}_2\text{-CH}_3$	1.33, 0.90
	B	$\text{=CH-CH}_2\text{-CH}_2\text{-CH}_2\text{-}$	2.01, 1.33
	C	$\text{-CH}_2\text{-CH}_2\text{-CH=CH-}$	2.02, 5.38
	D	$\text{-CH=CH-CH}_2\text{-CH=CH-}$	5.38, 2.84
	E	$\text{-O-C(O)-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-}$	1.62, 1.33
	F	$\text{-O-C(O)-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-}$	2.30, 1.60
	G (vicinal)	$\text{RO-CH}_2\text{-CH (OR)-CH}_2\text{-OR}$	4.12, 5.26
	G' (geminal)	RO-CHH'-CH-OR	4.09, 4.29
AMINO ACIDS			
Alanine	Ala	-CH-CH_3	3.79, 1.49
Glutamate	Glu	$\text{-CH-CH}_2\text{-CH}_2\text{-COO}^-$	2.21, 2.62
Isoleucine	Ile	$\text{-CH}_2\text{-CH}_3$	2.12, 0.97
Leucine	Leu	$\text{-CH-(CH}_3)_2$	1.78, 0.97
Lysine/polyamines	Lys	$\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-N}^+\text{H}_3$	1.72, 3.05
Threonine	Thr	-CH-CH (OH)-CH_3	4.27, 1.33
Valine	Val	$\text{-CH-(CH}_3)_2$	2.34, 1.03
CARBOHYDRATE MOIETIES			
Fucose	Fuc I	-CH-CH_3	4.27, 1.33
	Fuc II	-CH-CH_3	4.28, 1.25
	Fuc III	-CH-CH_3	4.30, 1.41
OTHER METABOLITES			
Inositol	Inos	-CH (OH)-CH (OH)	3.28, 3.64
Lactate	Lac	-CH (OH)-CH_3	4.12, 1.33
Taurine	Tau	$\text{H}_3\text{N}^+\text{-CH}_2\text{-CH}_2\text{-OSO}_3^-$	3.28, 3.50
Unassigned			3.96, 3.11
			3.76, 1.92
			4.22, 1.35
			3.63, 1.15

Table 2
Computerized Consensus Diagnosis of MRS on FNA

A Malignant vs Benign (SNR <25)

	Sensitivity	Specificity	%Crisp
Present	93%	92%	96%
Absent	92%	92%	95%

Overall Accuracy: 93%
Overall % crisp: 96%

B Malignant vs Benign (SNR >25)

	Sensitivity	Specificity	%Crisp
Present	94%	98%	100%
Absent	98%	94%	98%

Overall Accuracy: 96%
Overall % crisp: 99%

Table 2 continued

C Lymph Node Infiltration

	Sensitivity	Specificity	%Crisp
Present	96%	94%	90%
Absent	94%	96%	100%
Overall Accuracy: 95%			
Overall % crisp: 95%			

D Vascular invasion

	Sensitivity	Specificity	%Crisp
Present	84%	100%	94%
Absent	100%	84%	94%
Overall Accuracy: 92%			
Overall % crisp: 94%			

Table 3: Molecules identified in the ^1H MR COSY spectra of tissue specimens taken from primary breast lesions.

Molecules	Abbreviation	Coupling Partners (bold face)	Cross-peak Coordinates (ppm)	Number of crosspeaks				
				benign (n=8)	CIS (n=3)	malignant (n=4)		
LIPID								
Cholesterol	Z	CH ₂ -CH-ringα	0.85, 1.52	0	0	0		
Choline	Cho	(CH ₂) ₂ N ⁺ -CH ₂ -CH ₂ -OH	3.50, 4.07	1	0	0		
Glycerophosphocholine	Gro-P-Cho	(CH ₂) ₂ N ⁺ -CH ₂ -CH ₂ -OP(O)-O-	3.69, 4.38	1	0	0		
Phosphocholine	Cho-P	(CH ₂) ₂ N ⁺ -CH ₂ -CH ₂ -OPO ₃ ⁻	3.61, 4.25	0	0	0		
Triacylglycerol	A	-CH ₂ -CH ₂ -CH ₂ -CH ₃	1.33, 0.89	7	3	3		
	B	=CH-CH ₂ -CH ₂ -CH ₂ -	2.01, 1.33	7	3	3		
	C	-CH ₂ -CH ₂ -CH=CH-	2.01, 5.38	5	3	2		
	D	-CH=CH-CH ₂ -CH=CH-	5.38, 2.84	5	3	2		
	E	-O-C(O)-CH ₂ -CH ₂ -CH ₂ -CH ₂ -	1.62, 1.33	6	3	3		
	F	-O-C(O)-CH ₂ -CH ₂ -CH ₂ -CH ₂ -	2.30, 1.60	4	3	3		
	G (vicinal)	RC-CH ₂ -CH (OR)-CH ₂ -OR	4.12,5.26; 4.26,5.26	3	3	3		
	G' (geminal)	RC-CHH'-CH-OR	4.09, 4.29	6	3	2		
AMINO ACIDS								
Alanine	Ala	-CH-CH ₃	α-β	3.79, 1.49	2	0	0	
Glutamate/glutamine	Glu/Gln	-CH-CH ₂ -CH ₂ -COO ⁻	β-γ	2.10, 2.41	0	0	0	
Isoleucine	Ile	-CH-CH ₃	α-β	1.98, 1.02	2	3	1	
Leucine	Leu	-CH-(CH ₃) ₂	γ-δ	1.71, 0.97	2	0	1	
Lysine/polyamines	Lys	-CH ₂ -CH ₂ -CH ₂ -N ⁺ H ₃	δ-ε	1.72, 2.98	3	0	3	
Threonine	Thr	-CH-CH (OH)-CH ₃	β-γ	4.27, 1.33	2	1	1	
Valine	Val	-CH-(CH ₃) ₂	β-γ	2.34, 1.02	0	0	0	
CARBOHYDRATES								
Fucose	Fuc I	-CH-CH ₃		4.27, 1.33	2	1	1	
	Fuc II	-CH-CH ₃		4.28, 1.25	0	0	0	
	Fuc III	-CH-CH ₃		4.30, 1.41	1	0	0	
OTHER METABOLITES								
myc-Inositol	Ins	H5-H6	-CH (CH)-CH (OH)		3.25, 3.64	0	0	0
Lactate	Lac		-CH (CH)-CH ₃		4.12, 1.33	0	1	0
Taurine	Tau		H ₃ N ⁺ -CH ₂ -CH ₂ -		3.40, 3.26	1	0	0
Ethanol	EtOH		HO-CH ₂ -CH ₃		3.66, 1.18	1	0	2

Table 4: Molecules identified in the ^1H MR COSY spectra of FNA specimens taken from primary breast lesions.

Molecules	Abbreviation	Coupling Partners (bold face)	Cross-peak Coordinates (ppm)	Number of crosspeaks	
				benign (n=2)	malignant (n=14)
LIPID					
Cholesterol	Z	CH ₃ -CH-ringα	3.85, 1.52	0	5
Choline	Cho	(CH ₃) ₃ N ⁺ -CH ₂ -CH ₂ -OH	3.50, 4.07	0	4
Glycerophosphocholine	Gro-P-Cho	(CH ₃) ₃ N ⁺ -CH ₂ -CH ₂ -OP(O)-O-	3.69, 4.38	0	1
Phosphocholine	Cho-P	(CH ₃) ₃ N ⁺ -CH ₂ -CH ₂ -OPO ₃ ⁻	3.61, 4.25	0	5
Triacylglycerol	A	-CH ₂ -CH ₂ -CH ₂ -CH ₃	1.33, 0.89	2	14
	B	=CH-CH ₂ -CH ₂ -CH ₂ -	2.01, 1.33	2	13
	C	-CH ₂ -CH ₂ -CH=CH-	2.01, 5.38	1	8
	D	-CH=CH-CH ₂ -CH=CH-	5.38, 2.84	0	4
	E	-O-C(O)-CH ₂ -CH ₂ -CH ₂ -CH ₂ -	1.62, 1.33	2	14
	F	-O-C(O)-CH ₂ -CH ₂ -CH ₂ -CH ₂ -	2.30, 1.60	2	12
	G (vicinal)	RO-CH ₂ -CH (OR)-CH ₂ -OR	4.12,5.26; 4.26,5.26	0	3
	G' (geminal)	RO-CHH'-CH-OR	4.09, 4.29	0	8
AMINO ACIDS					
Alanine	Ala	-CH-CH ₃ α-β	3.79, 1.49	0	3
Glutamate/glutamine	Glu/Gln	-CH-CH ₂ -CH ₂ -COO ⁻ β-γ	2.10, 2.41	2	3
Isoleucine	Ile	-CH-CH ₃ α-β	1.98, 1.02	0	2
Leucine	Leu	-CH-(CH ₃) ₂ γ-δ	1.71, 0.97	0	2
Lysine/polyamines	Lys	-CH ₂ -CH ₂ -CH ₂ -N ⁺ H ₃ δ-e	1.72, 2.98	1	7
Threonine	Thr	-CH-CH (OH)-CH ₃ β-γ	4.27, 1.33	1	3
Valine	Val	-CH-(CH ₃) ₂ β-γ	2.34, 1.02	1	3
CARBOHYDRATES					
Fucose	Fuc I	-CH-CH ₃	4.27, 1.33	1	3
	Fuc II	-CH-CH ₃	4.28, 1.25	0	3
	Fuc III	-CH-CH ₃	4.30, 1.41	0	2
OTHER METABOLITES					
m/c-Inositol	Ins	H5-H6 -CH (OH)-CH (OH)	3.25, 3.64	0	2
Lactate	Lac	-CH (OH)-CH ₃	4.12, 1.33	1	11
Taurine	Tau	H ₃ N ⁺ -CH ₂ -CH ₂ -	3.40, 3.26	1	9
Ethanol	EtOH	HO-CH ₂ -CH ₃	3.56, 1.18	2	6

**MRS ON BREAST FINE NEEDLE ASPIRATE BIOPSY DETERMINES
PATHOLOGY, VASCULARISATION AND NODAL INVOLVEMENT**

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Bruce Barraclough, MBBS, FRACS; David Gillett, MD, FRACS;
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ABSTRACT

Aims: To use robust classification methods to analyse magnetic resonance spectroscopy (MRS) data of fine needle aspirates taken from breast tumours. The resultant data when compared with the histopathology and clinical criteria will provide computerised classification-based diagnosis and prognosis with a very high degree of accuracy and reliability.

Methods: Fine needle aspirate biopsies (FNAB), taken from 166 patients at time of surgery for diagnosis of benign and malignant breast diseases, were analysed by one-dimensional proton MRS at 8.5 Tesla. A sample of tissue was taken from around the needle tract for histopathological examination. Diagnostic correlation was performed between the spectra and standard synoptic pathology findings that contained detail regarding lympho-vascular invasion by the primary cancer and lymph node involvement of the excised axillary lymph nodes. The classification strategy consisted of three stages: pre-processing of MR magnitude spectra to identify optimal spectral regions, cross-validated Linear Discriminant Analysis, and classification aggregation via Computerised Consensus Diagnosis.

Results: Malignant tissue was distinguished from benign lesions with an overall accuracy of 93%. The false positives were all fibroadenomas. From the same spectrum lymph node involvement was predicted with an accuracy of 95% and tumour vascularisation with an overall accuracy of 92%.

Conclusion: The pathology, nodal involvement and tumour vascularisation can be determined by computerised, robust, statistical classification of the proton MRS spectrum from a FNAB taken from a primary breast lesion.

Key words: Breast neoplasms, pathology, magnetic resonance, mathematical analysis, nodal involvement.

INTRODUCTION

Clinical evaluation, mammography and aspiration cytology or core biopsy (triple assessment) is undertaken on women presenting with breast lesions in most Western countries. Clinical assessment of palpable breast lumps is unreliable ^{1, 2}. Impalpable lesions are usually discovered by screening or diagnostic mammography, which has a reported sensitivity of 77-94% and a specificity of 92-95% ³. Cytological assessment of fine needle aspiration biopsies (FNAB) has sensitivities ranging from 65-98% and specificities ranging from 34-100% ⁴ depending on the skill of the person performing the aspiration and the expertise of the cytopathologist.

Following surgical excision of the lesion a time consuming process of preparation and pathological assessment of the specimen determines the nature of the tumour and the prognostic features associated with it.

Magnetic resonance spectroscopy (MRS) is a modality with a proven record in the diagnosis of minimally invasive malignant lesions ⁵⁻¹¹. MR spectra of small samples of tissue or even cell suspensions enable the reliable determination of whether the tissue of origin is malignant or

benign. Often MRS is able to detect malignancy before morphological manifestations are visible by light microscopy ⁸.

The potential of proton MRS from FNAB specimens to distinguish benign from malignant breast lesions has been demonstrated previously ¹². At that time the MRS method relied on visual reading to process spectra and calculate the ratio of the diagnostic metabolites choline and creatine. This spectral ratio allowed tissue to be identified as either benign or malignant. In a small cohort of 20 patients within that study it also distinguished high grade ductal carcinoma *in situ* (DCIS) with comedonecrosis or microinvasion from low grade DCIS. Despite the limitation of visual inspection, which could only assess those spectra with a signal to noise ratio (SNR) of greater than 10 (*for definition of SNR see Methods: Data acquisition*), the visual method resulted in a diagnosis of malignant or benign with a sensitivity and specificity of 95 and 96%. Figure 1 shows malignant and benign spectra with good SNR while Figure 2 shows spectra with poor SNR.

Twenty percent of the spectra were discarded because low aspirate cellularity yielded inadequate

SNR. In the initial study visual analysis used only two of fifty or more available resonances⁶.

Thus, potentially diagnostic and prognostic information in the remaining spectrum may have been ignored.

A 3-stage, robust statistical classification strategy (SCS) has been developed to classify biomedical data and to assess the full MR spectrum obtained from biological samples. The robustness of the method has been demonstrated previously with the analysis of proton MR spectra of thyroid tumours¹³, ovarian¹⁴, prostate⁹, and brain tissues¹⁵. We have applied SCS to assess proton MR spectra of breast aspirates against pathological criteria in order to determine the correct pathology on samples with sub-optimal cellularity and SNR and to determine if other diagnostic and prognostic information is available in the spectra.

We report that SCS on MRS from breast FNAB is more reliable than visual inspection to determine whether a lesion is benign or malignant, and that a greater proportion of spectra is useful for analysis. Furthermore, spectral information obtained from MRS on FNAB of breast cancer specimens predicted lymph node metastases (overall accuracy of 96%) and vascular

invasion (overall accuracy of 92%).

METHODS

Patients:

Intra-operative FNAB were taken from 139 patients undergoing breast surgery for malignant and benign conditions (Table 1) by three surgeons in separate hospitals from January 1994 until December 1995. In order to provide a sufficiently large data set for SCS an additional 27 patients joined the study (see Table 1) from 1998 to mid 1999.

Impalpable breast lesions that had been localised by carbon track or hook wire were included, but removed from the study if the lesion was not palpable at excision or when the pathology specimen could have been compromised. All samples were taken during surgery under direct vision after the lesion had been identified and incised sufficiently widely to ensure that the FNAB and tissue specimens represented the same lesion and were thus comparable. The lesion was identified and incised *in-vivo* via the margin with the greatest depth of normal tissue between it and the lesion to ensure the pathologist could report upon the lesion according to a standard protocol. Malignant and suspicious lesions were orientated with sutures and radio opaque vascular clips (Ligaclips) for pathological and radiological orientation. The FNAB was collected

by the surgeon using a 23-gauge needle on a 5ml syringe. The number of needle passes was recorded and the surgeon's evaluation of the quality of the aspirate was made. Before the needle was removed from the lesion, a tissue sample including the relevant part of the needle track was taken. The size of this tissue specimen was estimated and recorded by the surgeon.

Pre-operative clinical and investigative data included localised pain, nipple discharge or nipple crusting, details of previous mammography, and whether the lesion was detected through screening. The clinical, mammography, ultra sonographic, cytological, core biopsy and MRI details were recorded as malignant, suspicious, benign, impalpable, uncertain or not done.

The pathology specimen was sent on ice at the initial stages, but later in formalin, for standard histopathological reporting and hormone receptor analysis. The pathology report was issued in synoptic format ¹⁶.

Specific tumour-related clinical and sampling information was collected. These included a history of previous breast biopsies with dates, diagnoses, sizes and sites of these lesions along

with the current lesion's duration, palpability, laterality, size and locality within the breast. The date of operation, the extent of breast surgery from open biopsy to total mastectomy, and axillary surgery from sampling to level 3 dissection was recorded.

Specimen preparation: Following complete excision of the lesions the FNAB cytology and tissue specimens were placed in polypropylene vials containing 300ml phosphate-buffered saline (PBS) in D₂O. All specimens were immediately immersed in liquid nitrogen and stored at -70°C for up to 6 weeks until MRS analysis.

Prior to the proton MRS experiment, each FNAB specimen was thawed and transferred directly to a 5 mm MRS tube. The volume was adjusted to 300ml with PBS/D₂O where necessary. Proton MRS assessment of all specimens was performed without knowledge of the correlative histopathology, either from the synoptic pathology report or from sectioning of tissue used in MRS study.

The sample of tissue excised around the needle tract was similarly placed in polypropylene vials

containing 300ml PBS/D₂O and immersed in liquid nitrogen as described above. This sample was later used for pathological correlation.

Data acquisition: MRS experiments were carried out on a Bruker Avance 360 wide-bore spectrometer (operating at 8.5 Tesla) equipped with a standard 5 mm dedicated proton probehead. The sample was spun at 20 Hz and the temperature maintained at 37°C. The residual water signal was suppressed by selective gated irradiation. The chemical shifts of resonances were referenced to aqueous sodium 3-(trimethylsilyl)-propanesulphonate (TSPS) at 0.00 ppm. One-dimensional spectra were acquired over a spectral width of 3597 Hz (10.0 ppm) using a 90° pulse of 6.5 - 7 µs, 8192 data points, 256 accumulations and a relaxation delay of 2.00 seconds, resulting in a pulse repetition time of 3.14 seconds.

SNR was determined using the Bruker standard software (xwinnmr). The noise region was defined between 8.5 to 9.5 ppm. The signal region was defined between 2.8 to 3.5 ppm

Histopathology: Diagnostic correlation was obtained by comparing spectral analysis with the

hospital pathology report provided for each patient. Lymph node involvement and vascular invasion were determined from the reports only in cases where this information was complete.

In the participating hospitals lymph nodes were embedded and serial sectioned in standard fashion. One 5µm section out of every 50 (ie each 250µm) was stained and examined. All intervening sections were discarded.

In the initial phase of the study, cytological analysis of the aspirate after MRS analysis was attempted but cellular detail was compromised by autolytic changes and this approach was not pursued. In order to verify FNAB sampling accuracy, a separate histopathological assessment by a single pathologist (PR) was obtained from tissue removed from the aspiration site of the MRS sample (see *Specimen preparation*). Tissue specimens were thawed, fixed in FAA (formalin/acetic acid/alcohol), paraffin-embedded, sectioned at 7 µm, stained with haematoxylin and eosin according to standard protocols and reviewed under the light microscope by the pathologist without access to the clinical or MRS data. Tissue preservation, abundance of epithelial cells relative to stroma, and presence of potentially confounding factors such as fat and inflammatory cells were reported in addition to the principal diagnosis.

SCS: The general classification strategy developed at the Institute for Biodiagnostics (IBD) was designed specifically for MR and IR spectra of biofluids and biopsies. The strategy consists of three stages. First the MR magnitude spectra are preprocessed, (in order to eliminate redundant information and/or noise) by submitting them to a powerful genetic algorithm-based Optimal Region Selection (ORS_GA) ¹⁷, which finds a few (at most 5-10) maximally discriminatory subregions in the spectra. The averages in these subregions are the ultimate features and used at the second stage. This stage uses the features found by ORS_GA to develop Linear Discriminant Analysis (LDA) classifiers that are made robust by IBD's bootstrap-based crossvalidation method ¹⁸. The crossvalidation approach proceeds by randomly selecting about half the spectra from each class and using these to train a classifier (usually LDA). The resulting classifier is then used to validate the remaining half. This process is repeated B times (with random replacement), and the optimized LDA coefficients are saved. The weighted average of these B sets of coefficients produces the final classifier. The ultimate classifier is the weighted output of the 500-1000 different bootstrap classifier coefficient sets and was designed to be used in a clinical setting as the single best classifier. The classifier consists of probabilities of class assignment for the

individual spectra. For particularly difficult classification problems the third stage is activated.

This aggregates the outputs (class probabilities) of several independent classifiers to form a

Computerised Consensus Diagnosis (CCD) 13, 15. The consequence of CCD is that

classification accuracy and reliability is generally better than the best of the individual classifiers.

RESULTS

One hundred and sixty-six patients were involved in the study. A summary of the clinicopathological criteria is shown in Table 1.

Benign versus Malignant: Proton MR spectra were recorded for each FNAB irrespective of the cellularity of the aspirate. However, those specimens with a SNR less than 10, which in our previous report were shown to be inadequate for visual inspection¹² have been included in the SCS analysis without significantly compromising accuracy. Visual inspection of all spectra irrespective of signal to noise gave a sensitivity and specificity of 85.3% and 81.5% respectively (Table 2), based on the creatine-to-choline ratio. When SCS-based classifiers were developed for all available spectra (Table 3), 96% of the spectra were considered crisp and could be assigned unambiguously by the classifier as malignant or benign. Sensitivity and specificity were 92% respectively.

Removing the 29 spectra with the previously determined poor SNR ($\text{SNR} < 10$) a sensitivity and specificity of 98% and 94% respectively was achieved with crispness of 99%

Prognostic factors: With the addition of prognostic criteria to the database two further classifiers were created namely lymph node involvement and vascular invasion. A small number of known benign or pre-invasive cases were included in these subsets to assess the computer's ability to correctly define those cases in which no nodal involvement or vascular invasion was expected. These benign or pre-invasive cases were all correctly assigned by the computer into their respective uninvolved classes.

Lymph node Involvement: There were 31 cases with nodal involvement and 30 without including 2 DCIS and 3 fibrocystic specimens. All spectra were included irrespective of SNR. Only those spectra for which complete pathology and clinical reports were available were included in this comparison (Table 1). The presence of lymph node metastases was predicted by SCS with a sensitivity of 96% and specificity of 94% (Table 4).

Vascular Invasion: SCS-based analysis of spectra was also carried out using vascular invasion as the criterion. There were 85 spectra for this analysis (Table 1). A sensitivity of

84% and specificity of 100% was achieved for the correct determination of vascular invasion, with an overall accuracy of 92% (Table 4).

DISCUSSION

The introduction of preprocessing and SCS analysis of MR spectra has enhanced the ability to correlate spectroscopic changes with the pathology of human biopsies. It has also allowed specimens with sub-optimal cellularity to be analysed, and more importantly, provided a correlation with clinical criteria not apparent by visual inspection.

Visual inspection of spectra, like histopathology, is limited by the experience and skill of the reader for determining peak height ratios of metabolites ¹². Visual inspection of spectra and the use of peak height ratio measurements of choline and creatine discriminated benign from malignant spectra with a higher degree of accuracy than standard triple assessment of breast lesions. However, to attain a high degree of accuracy, many spectra with poor SNR had to be discarded, reducing the effectiveness of the technique. Previous estimates of cellular material derived from FNAB, on which to perform MRS analysis reliably, have suggested that at least 10^6 cells are needed ⁶.

By using SCS-derived classifiers it was possible to distinguish malignant from benign pathologies with higher sensitivity 92% and specificity 96% for all FNAB spectra including those with low SNR (Table 3) than by visual reading of these same spectra (Table 2). That SCS-based analysis could more reliably classify a greater proportion of spectra than could be visually assessed is testament to the robustness and greater generality of the computer-based approach.

The SCS-based result is further improved by presenting to the computer spectral data with high SNR. The improvement in sensitivity and specificity gained for spectra with $SNR > 10$ (Table 3) illustrates this point and supports the need for appropriate training of clinical personnel to obtain FNAB with adequate cell numbers.

SCS has allowed us to train classifiers to recognise patterns containing more complex information. We have trained and subsequently validated the classifier to diagnose specimens with lymph node involvement and vascular invasion. The ability of the SCS-derived classifier to predict lymph node involvement with an accuracy of 95% and vascular invasion with an

accuracy of 92% emphasises the wealth of chemical information that can be extracted, with the appropriate statistical approach, from an FNAB of a breast lesion (Table 3).

A major challenge in breast cancer is the need to identify and understand the factors that most influence the patient's prognosis and through timely and appropriate intervention influence this outcome. Adjuvant therapy can reduce the odds of death during the first ten years after diagnosis of breast cancer by about of 20-30% ¹⁹. The best prognostic indicator of survival in patients with early breast cancer has been shown to be axillary lymph node status ²⁰⁻²².

Increasingly, sentinel lymph node biopsy is being investigated as a means to reduce the morbidity and cost of unnecessary axillary dissection in the two thirds of women with early invasive breast cancer who prove to be node-negative ²³⁻²⁵, while preserving the option of full axillary node clearance in those patients who are node-positive. If MRS can determine nodal involvement from the cellular material derived solely from the primary tumour, the role of sentinel lymph node biopsy will require further scrutiny.

The present report, that 52% of patients with lymph node involvement also had vascular invasion, is in agreement with Barth et al ²⁶, who showed that peritumoural lympho-vascular invasion correlated with lymph node involvement ²⁷ and was an independent predictor of disease free and overall survival ²⁸⁻³¹.

A computer-based statistical classification strategy providing a robust means of analysing clinical data is becoming a reality. The power, speed and reproducibility of a computer-based diagnosis may lead to the expectation that suitably programmed computers will supplant the human observer in the clinical laboratory. Patients increasingly expect certainty in diagnosis and optimum management.

However, caution is warranted. Several important experimental factors need to be considered. The MRS method only works on aspirated cells from the breast and not on core biopsies that contain a sufficiently high level of fat to mask diagnostic and prognostic information. The method will only be successful if the biopsy is representative of the lesion and contains sufficient cellularity. Furthermore, sample handling is of paramount importance if the specimen is to be

minimally degraded. There must be quality control in the spectrometer with regard to pulse sequences, temperature, magnet stability, shimming and water suppression. The magnetic field at which this database was collected is 8.5 Tesla (360MHz for proton) and it must be emphasised that spectral patterns are frequency dependent. Therefore a new classifier must be developed if one uses different magnetic field strengths.

Finally, unless the clinical and pathology databases used to train the classifier are representative of the full range of pathologies or the complete demographics of the population, the classifier will be inadequately prepared for all the possibilities it might encounter in clinical practice. In developing a database for breast lesions, it is necessary to ensure that the training set has adequate samples of all the commonly encountered breast pathologies and to update continually the training set when less common tumour types are detected.

Let not the note of caution undermine the revolutionary impact on breast cancer management that has come about by the use of SCS computerised analysis of MR spectral features. It has revealed a much higher level of accuracy in diagnosis of the lesion and also an indication of its metastatic

potential when compared to visual inspection of spectra. Most importantly, it has facilitated the staging of the disease from spectral information of FNAB collected only from the primary breast lesion.

CONCLUSION

Pathological diagnosis, the likelihood of axillary lymph nodal involvement and tumour vascularisation can be determined by SCS-based analysis of proton MR spectra of a FNAB taken from a primary breast lesion. The SCS-based method is more accurate and reliable than visual inspection for identifying complex spectral indicators of diagnosis and prognosis.

The ability of an SCS-based analysis of MRS data to provide prognostic information on lymph node involvement by sampling only the primary tumour may provide a paradigm shift in the management of breast cancer. The determination of vascular invasion from the same cellular material highlights the untapped potential of MRS to determine prognostic information.

ACKNOWLEDGEMENTS

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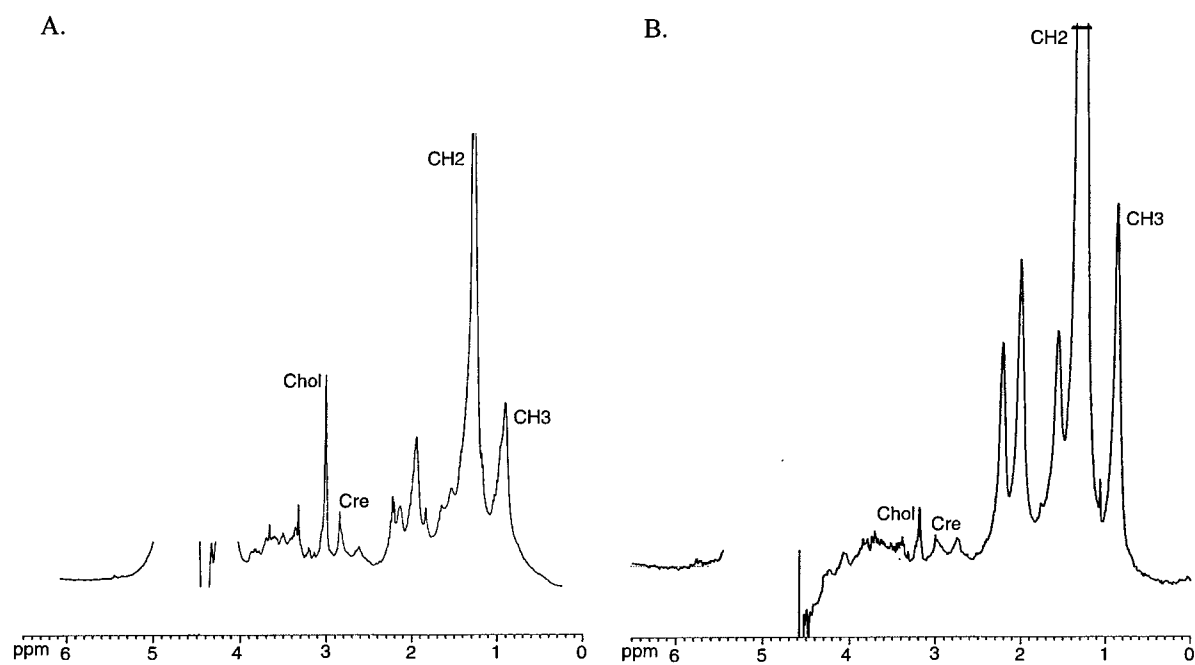
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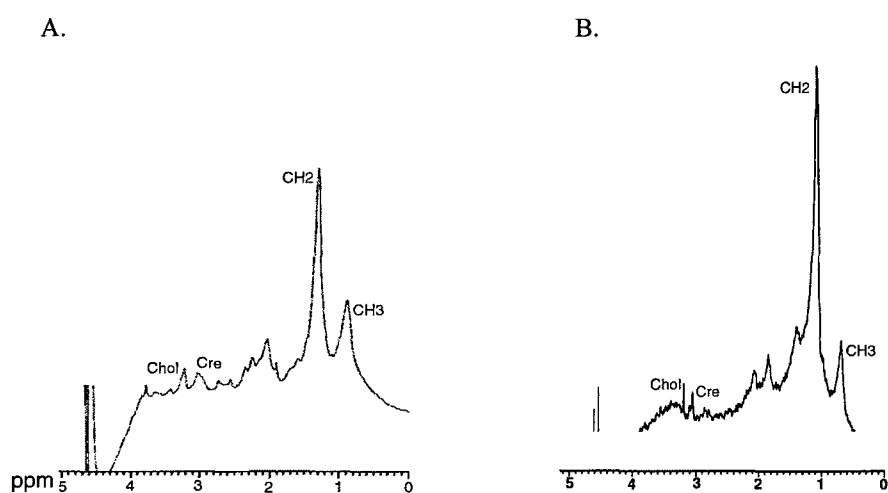
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Figure 1.



MR Spectra with SNR>10. (A) Malignant, (B) Benign
For acquisition parameters see Materials and Methods.
A line broadening of 3Hz was applied to both spectra.
The spectra were displayed in absolute intensity mode
(noiselevel similar in both spectra).

Figure 2.



MR Spectra with SNR<10. (A) Malignant, (B) Benign
For acquisition parameters see Materials and Methods.
A line broadening of 3Hz was applied to both spectra.
The spectra were displayed in absolute intensity mode
(noiselevel similar in both spectra).

Table 1
Summary of Clinico-pathological Data

	All patients (n=166)	Benign/Malignant (n=139)	Lymph Nodes (n=61)	Vascular Invasion (n=83)
Age Mean±SD (Range)	55.8±15.4 (20-101)	54.7±15 (20-90)	58.4±13.2 (29-85)	60.6±14.3 (29-101)
Pathology type				
Invasive Ductal	89	73	52	66
Invasive Lobular	8	8	3	5
Mixed Ductal/ Lob.	1	1	1	
DCIS	10	1	2*	9*
Fibroadenoma	17	17		
Fibrocystic	22	22	3*	2*
Papilloma	3	2		
Radial Scar	2	2		
Gynaecomastia	1	1		
Misc. Benign	13	12		1
Total	166	139	61	83
Nodal Status				
Positive			31	
Negative			30	
Total Nodes			14.2±7.9 (1-34)	
No. Positive Nodes			2.2±4.3 (0-28)	
Vascular Invasion				
Present				32
Absent				51

* These preinvasive and benign lesions were included as known lymph node negative, vascular invasion negative cases to test the computer's ability to discern true negatives and positives. They were all correctly classified by the computer into their respective classes.

Table 2
Visual Inspection:- Malignant vs. Benign

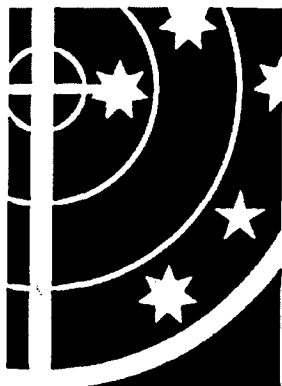
	Sensitivity	Specificity
For all Spectra		
Malignant (n=84) vs. Benign (n=55)	85.3%	81.5%
Spectra with SNR>10		
Malignant (n=62) vs. Benign (n=48)	100%	87.3%

Table 3
SCS:- Malignant vs. Benign

	Sensitivity	Specificity	% Crisp	Accuracy
For all Spectra				
Malignant (n=84) vs. Benign (n=55)	92%	92%	96%	93%
Spectra with SNR>10				
Malignant (n=62) vs. Benign (n=48)	98%	94%	99%	96%

Table 4
SCS:- Prognostic Indicators

	Sensitivity	Specificity	% Crisp	Accuracy
Lymph Node Involvement				
Present (n=31) vs. Absent (n=30)	96%	94%	95%	95%
Vascular Invasion				
Present (n=32) vs. Absent (n=51)	84%	100%	94%	92%



INSTITUTE
FOR
MAGNETIC
RESONANCE
RESEARCH
— SYDNEY —

**WORKSHOP ON MAGNETIC RESONANCE SPECTROSCOPY
OF THE BREAST**

Thursday 2nd and Friday 3rd March 2000
The Blaxland
Blaxland Road
Ryde

PROGRAM

Session 1 – Thursday 2 March

Time	Topic	Presented by
10.00am – 10.30am	Registration/Morning tea	
10.30am – 10.45am	Welcome by the Chairpersons	Dr Carolyn Mountford, IMRR Dr Peter Malycha, Royal Adelaide Hospital/IMRR
10.45am – 11.15am	Breast Cancer Management in Australia	Dr Peter Malycha Royal Adelaide Hospital/IMRR
11.15am – 11.45am	The IMRR	Dr Carolyn Mountford, IMRR
11.45am – 12.15pm	The Karolinska Institute	Dr Bertil Hamberger Karolinska Institute
12.15pm – 1.00pm	Breast Cancer Management in Sweden	Dr Kerstin Sandelin Karolinska Institute
1.00pm – 2.00pm	Lunch	
2.00pm – 2.30pm	The Beth Israel and Deaconess Hospitals	Dr Herbert Kressel Beth Israel and Deaconess Hospitals, Harvard Medical School
2.30pm – 3.00pm	Breast Cancer Management in the United States	Dr Laurence Gluch, IMRR
3.00pm - 3.30pm	Institute for Biodiagnostics, National Research Council	Dr Ian Smith National Research Council of Canada
3.30pm – 3.45pm	Afternoon Tea	
3.45pm – 4.15pm	MRI – What it can/can't do for the Breast	Dr Herbert Kressel Beth Israel and Deaconess Hospitals, Harvard Medical School
4.15pm – 4.45pm	What do Surgeons really want from MR?	Dr David Gillett Concord Hospital/IMRR
4.45pm – 5.15pm	Why Pathologists need MRS	Prof Peter Russell Royal Prince Alfred Hospital/IMRR
5.15pm – 6.30pm	Pre Dinner Drinks	The Blaxland Terrace

Session 2 – Friday 3 March

Time	Topic	Presented by
8.30am – 9.00am	MRS and FNAB	Dr Cynthia Lean, IMRR
9.00am – 9.30am	Statistical Computerised Strategy Methods	Dr Ian Smith National Research Council of Canada
9.30am – 10.00am	<i>In Vivo</i> Spectroscopy Breast	Dr Carolyn Mountford, IMRR
10.00am – 10.30am	Practical Aspects of FNAB for MRS and Patient Record Keeping	Dr Peter Malycha Royal Adelaide Hospital/IMRR
10.30am – 11.00am	Technical Aspects of Spectex	Dr Uwe Himmelreich, IMRR
11.00am – 11.15am	Morning Tea	
11.15am – 11.45am	Ductal Carcinoma "in situ"	Dr Michael Bilous Westmead Hospital
11.45am – 1.00pm	Open Discussion on the Protocol currently used by the IMRR – Is it suitable for the United States and Sweden?	
1.00pm – 1.30pm	Lunch	
1.30pm – 1.45pm	Closing Remarks	Emeritus Professor Tom Reeve, AC, CBE Royal North Shore Hospital Australian Cancer Network

Contact Numbers for The Blaxland:

Phone: 1300 366 835
Facsimile: 1300 366 835

Multi-Centre International Trial

Magnetic Resonance Spectroscopy of Fine Needle Aspiration Biopsies of the Breast

Participation Protocol

Patient Identification

- Unique identifier: First three letters of surname, Date of Birth: DDMMYYYY eg. MAL15111944
- Use only current family name (surname)
- Name and sample type on ground glass of vial
- MRS sample details recorded in operation report

Clinical Information to be Recorded in Patient's Notes

- Presenting symptoms
- Method of diagnosis
- Medical history
- Family history
- Pregnancies and breast feeding
- Hormone exposure
- Menstrual history

Collection of FNA Specimens for MRS

- Breast lesion identified at operation
 - Mobilised to ensure margins adequate
 - Cancer opened via maximum margin
 - Usually whilst attached to blood supply
 - 23 gauge needle via skin into lesion
 - 23 gauge needle via wound into same site
 - 12 - 20 passes with suction/5ml syringe
 - Contents of needle expelled and rinsed in PBS/D₂O vial
 - All samples snap frozen immediately in liquid nitrogen
 - Excision of needle track (about 1cm³ of tissue): direct vision
- Sample 1: Labelled "FNA skin"
Sample 2: Labelled "FNA at op"

Sample 3: Labelled "tissue"

Operation Note

- Standard operation detail
- MRS information
- FNAB via skin
- FNAB from lesion at operation
- Tissue sample from lesion

Cancers Excluded from MRS

- Less than 5-7mm where pathology specimen could be compromised
- impalpable lesions at operation
- where margin of clearance could be compromised

Pathology Report

- Synoptic reporting essential (see attached copy)
- HRA
- MIB, Her2 optional etc

Transport of Specimens

- Specimens placed in liquid nitrogen
- sent to IMRR within 6 weeks
- paper work mailed at same time
- date of transport noted
- record of all MRS specimens kept in surgeon's office

CME ARTICLE

Magnetic Resonance Spectroscopy A New Gold Standard for Medical Diagnosis?

Cynthia L. Lean, PhD,* Peter Russell MD, FRCPA,†‡ and Carolyn E. Mountford, DPhil§

Magnetic resonance spectroscopy (MRS) can greatly facilitate the management of malignant disease in the new millennium by providing more accurate diagnosis and prognostic information that can not be obtained using current modalities. Key women's health issues can be improved using MRS, including the management of cervical cancer, thyroid cancer, and breast cancer.

Combined with mathematical analysis of spectral data using statistical classification strategies, MRS can provide diagnostic information from patient cell samples to identify a malignant neoplastic process with an accuracy approaching 100%. During the early years of technology development and data acquisition, and until the robustness of MRS technology is proven, this accuracy is dependent on precise and careful correlation of MRS and histologic diagnoses of the tissue sample.

Magnetic resonance spectroscopy is poised to supplement the already significant contribution of magnetic resonance imaging to the clinical management of disease, including cervical cancer, thyroid cancer, and breast cancer. Spectroscopy *ex vivo* can identify important subsets of disease entities that are not morphologically manifest, whereas spectroscopy *in vivo*, if capable of sensitivities and specificities comparable with those obtained on excised biopsy specimens, could provide noninvasive localization and diagnosis of tumors. To ensure rapid entry of these technologies into clinical practice, the spectroscopy community must be aware of its responsibility to produce national and international trials and healthcare outcomes. [Key words: breast cancer, cervical cancer, magnetic resonance spectroscopy, pathology] *Journal of Women's Imaging* 2000;2:19-30

Learning Objectives: After reading this article and completing the post-test, the physician should be able to

- describe the technique of magnetic resonance spectroscopy;
- describe how the use of magnetic resonance spectroscopy provides unique and prognostic information

for cervical cancer, follicular thyroid cancer, and breast cancer;

- identify the ways in which the use of magnetic resonance spectroscopy could benefit women's health in different regions of the world, depending on the available medical resources and the clinical problems endemic to specific patient cohorts.

Magnetic resonance spectroscopy (MRS) will assist in reshaping medical diagnosis in the new millennium. The method will provide a fast, accurate, and robust diagnosis of human disease, and will remove the complexity from, and perhaps partly replace, orthodox histopathology. Women's health will benefit from this progress, particularly with respect to neoplastic conditions that are difficult to diagnose accurately using current modalities.

Histopathology has been the gold standard in the 20th century, providing the final and definitive diagnosis of invasive and preinvasive human malignancy; however, as a diagnostic modality, it has limitations. These limitations have been apparent from the observation that clinical outcomes often differ from outcomes predicted by histopathology. Histopathologic analysis is limited because there is a continuum of changes that may make precise diagnosis of cancer difficult. The skill, experience, and thoroughness of surgeons and pathologists are required to ensure the suitability of a given biopsy specimen for histopathologic assessment and from which detailed diagnostic and prognostic clues are to be gleaned. Further, sampling errors are inherent; from any biopsy specimen, usually less than 1% of the tissue is routinely assessed.

During the past 20 years, and following the report on tumor heterogeneity by Fidler and Kripke,¹ it has become evident that tumor development and progression involves a continuum of chemical changes that reflect a variety of biologic events and clinical situations. Many diseases are multistep processes, and histopathology cannot always discern all of the steps involved; biologic changes that are not manifested morphologically can occur.

The clinical application of MRS to the detection, diagnosis, and management of malignant disease has been slow to emerge. For many years, MRS was considered to be a method that was unable to correctly discern tissue changes. On the contrary, MRS was able to accurately

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report tissue changes. What early studies lacked was a concerted effort to analyze MRS data against either known clinical outcome or careful pathologic assessment of the entire tissue specimen examined using MRS.

■ Comparison of Proton MRS Profiles with Histopathology and Clinical Outcome

Different chemical species are of variable diagnostic importance for each organ and pathologic state,^{2,3} and magnetic resonance-visible chemistry reflects biologic events that are either completed or in progress. More than 50 chemical species can be measured simultaneously using proton MRS during tumor development and progression; however, the potential use of these chemical signatures for diagnostic and prognostic purposes must be investigated in patient cohorts that are large enough to accommodate through statistical analysis.^{2,3}

We have established that proton MRS *ex vivo* can diagnose or exclude epithelial malignancy with at least, if not more than, the accuracy of current aspiration or exfoliative cytologic techniques used for the cervix,⁴ colon,⁵ ovary,⁶ breast,⁷ and thyroid.^{8,9} The acute sensitivity of the MRS method was illustrated in the detection of microscopic metastatic deposits in the lymph nodes of tumor-bearing rats.¹⁰ These micrometastases were missed by detailed histopathologic analysis, but were confirmed by xenografting the nodes to hairless mice and recording subsequent tumor growth.

A key area of diagnostic medicine that will benefit from proton MRS is women's health, which varies among countries, cultures, and environments. What is of clinical concern to one community may not be of importance to another; a disease process can have different clinical ramifications according to prevalence, dominant living standards, and the presence of early screening programs. Diagnostic and screening procedures involving spectroscopy that address the clinical problems facing patient cohorts in different regions and the costs and benefits of applying this technology in societies with access to different resources are needed.

This review examines the current status of MRS research and the potential for MRS to influence diagnosis and management of cervical cancer, follicular thyroid disease, and breast cancer during the next decade.

■ Cervical Cancer

Cancer of the uterine cervix is the second most common malignancy in women worldwide.¹¹ Screening programs to decrease the incidence of this disease assume that cervical cancer is preceded by preinvasive neoplastic stages, which are generally termed cervical intraepithelial neoplasia (CIN) or, more recently, squamous intraepithelial lesions (SIL).

The diagnosis of cervical cancer currently relies on histologic examination of the tissue obtained during colposcopy, but this process is not problem-free. Sampling errors made by the gynecologist can be introduced at the

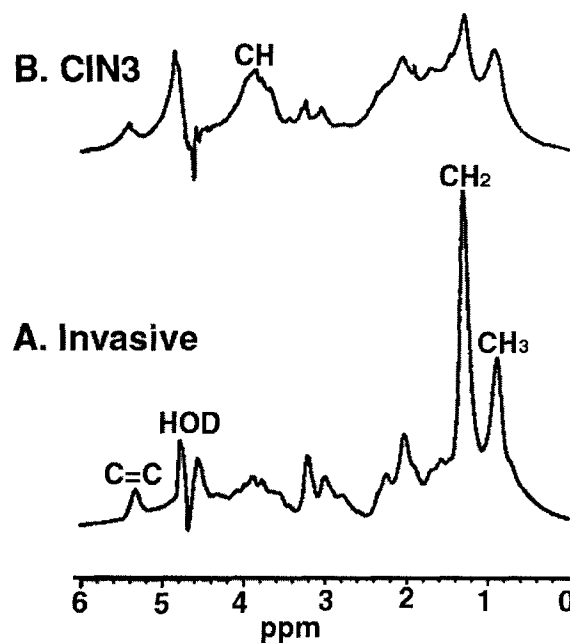


Figure 1. One-dimensional proton magnetic resonance spectra of cervical punch biopsy specimens; **A**, frankly invasive carcinoma, and **B**, cervical intraepithelial neoplasia grade 3 carcinoma *in situ*. =C = olefinic protons; HOD = residual water signal. Reprinted with permission from the Radiological Society of North America.⁴

time of biopsy, only to be compounded by processing artifacts in the laboratory and subjective pathologic assessment of the section. By definition, the *in situ* phase of an epithelial malignancy contains cells that are morphologically indistinguishable from cells in the invasive state; therefore, the diagnosis of invasive cancer rests not on cytologic criteria, but on histologic evidence of destructive invasion. More importantly, the pathologic changes can only be used to statistically predict the patient's clinical course; thus, most patients are either undertreated or overtreated. Standardization of treatment protocols is introduced to minimize the level and consequences of treatment error.

Spectroscopy Ex Vivo

In our original study, one-dimensional proton MR spectra were obtained from 40 specimens of invasive carcinoma and from 119 preinvasive specimens.⁴ Spectra of 39 of 40 invasive specimens (Figure 1A) were characterized by an intense resonance at 1.3 ppm, which arose primarily from methylene protons of acyl chains in mobile neutral lipid, with additional contributions from the methyl protons of lactate and threonine.

The spectra from high-grade carcinoma *in situ* (CIS/CIN 3) specimens (Figure 1B) displayed methyl, $^{15}\text{N}(\text{CH}_3)_3$, and olefinic resonances at 0.9 ppm, 3.2 ppm, and 5.2 ppm, respectively. In addition, there were prominent resonances at 1.7 ppm, 2.0 ppm, and 3.0 ppm; however, these preinvasive specimens lacked the intense methylene resonance at 1.3 ppm. The other visible spectral difference was increases in the broad, featureless resonance between 3.4 ppm and 4.2 ppm in preinvasive

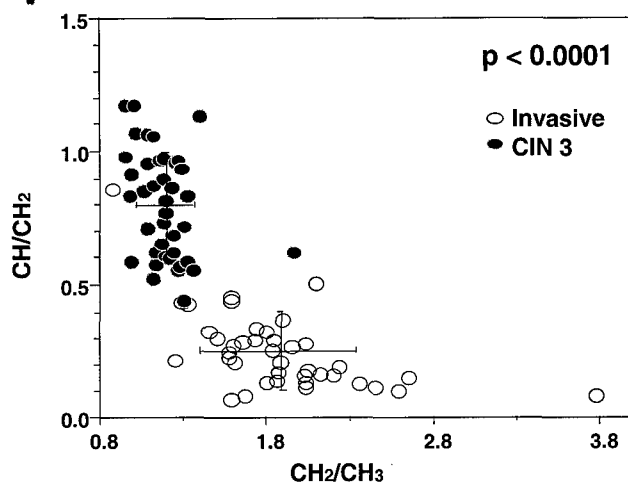


Figure 2. Peak height ratios from one-dimensional magnetic resonance spectra. Two ratios are plotted on the same graph: the ratio of the CH/CH_2 resonances versus the ratio of the CH_2/CH_3 resonances. The graph compares grade 3 carcinoma *in situ*, one of the higher-grade dysplastic states, with invasive carcinoma. The cross hairs show the mean \pm SD for each category. A *t* test gives $P < 0.0001$ for CH/CH_2 and CH_2/CH_3 . Reprinted with permission from the Radiological Society of North America.⁴

specimens, compared with their invasive counterparts. This latter region, which arises mainly from protons on carbohydrate, protein, and phospholipid metabolites, is denoted as the CH resonance.

When the CH_2/CH_3 ratio was plotted against the CH/CH_2 ratio to compare the premalignant states with invasive carcinoma (Figure 2), a separation between invasive and preinvasive cells was achieved with a sensitivity and specificity of 94% and 98%, respectively.⁴ Thus, proton MRS was able to distinguish between preinvasive and invasive cervical cancer *ex vivo*, based on the detection of altered invasive-cell chemistry.

A simple MRS experiment examines the entire biopsy specimen and leaves the tissue intact for further histologic assessment. The National Research Council in Winnipeg substantiated these data with an independent study.¹²

Spectroscopy *In Vivo*

With the ready availability of the Papanicolaou test in developed countries (a test that provides fast, cheap, and relatively reliable screening for cervical neoplasms with a moderate sensitivity and high specificity) the clinical relevance of these magnetic resonance *ex vivo* data resides not in screening, but in providing the basis of the development of *in vivo* spectroscopic protocols. These protocols would enable reliable, noninvasive diagnosis of established cancers, particularly in countries where cervical screening is not well established and many women present with late-stage disease.

In Korea, where the first successful *in vivo* spectroscopy of the uterine cervix was undertaken (Asan Medical Center, Seoul, Korea¹³), cervical cancer remains the most prevalent female cancer. Despite a reduction in the dis-

ease from 32% of the female population due to the implementation of widespread screening programs, cervical cancer affects 20% of the female population. Many Korean women present with large, invasive cancers of the cervix. For these patients, *in vivo* MRS has the potential to significantly improve treatment by providing simultaneous diagnosis and staging of the lesion before surgery.

Proton magnetic resonance spectra from healthy human cervical cells collected at 1.5 T *in vivo* do not exhibit magnetic resonance signals greater than the noise level. Conversely, the spectrum of invasive cervical cancer is comparable with the spectrum obtained *ex vivo* with resonances from creatine (3.0 ppm), choline (3.2 ppm), and lipid (1.3 ppm) apparent. Spectra were obtained from cells of invasive squamous cell carcinomas and a cervical adenocarcinoma. Resonances from choline and creatine were observed in the spectra of both lesions, whereas the triglyceride resonance was present only in the spectrum of the squamous cell cancer.¹³

These preliminary data are promising, and with the *ex vivo* cervical data support the hypothesis that proton MRS has the ability to provide diagnosis and staging of cervical lesions *in vivo* before surgery. In some centers, water-based magnetic resonance imaging for the preoperative assessment of patients with clinical cervical cancer is advocated.¹⁴⁻²⁰ Because MRS can accurately diagnose cervical malignancy *ex vivo*, *in vivo* spectroscopy or spectroscopic imaging can materially enhance the preoperative assessment of the primary tumors and the status of the draining lymph nodes before surgery.

■ Follicular Thyroid Adenoma

Thyroid nodules are common, particularly in women, and are clinically evident in up to 10% of the population. Although the majority (90-95%) of solitary thyroid nodules are benign,^{21, 22} the exclusion of malignancy in follicular thyroid nodules remains a significant diagnostic problem; the diagnosis is currently based on the excised lesion obtained during partial thyroidectomy. Preoperative fine needle aspiration biopsy (FNAB) cytology, although accurate in identifying papillary, medullary, and anaplastic carcinoma, can not reliably distinguish benign and malignant follicular neoplasms.

Histologically, tumors that are arbitrarily designated as follicular adenomas and follicular carcinomas are indistinguishable in clinical, radiologic, and gross pathologic features, and one must rely on detection of capsular or vascular invasion at the periphery of the neoplasm or metastases to identify the carcinomas. As noted previously, this analysis requires surgical removal of the entire tumor and extensive laboratory examination.

Spectroscopy *Ex Vivo*

In a study of 53 consecutive patients with thyroid nodules, we established that one-dimensional proton MRS could distinguish normal thyroid tissue from all types of

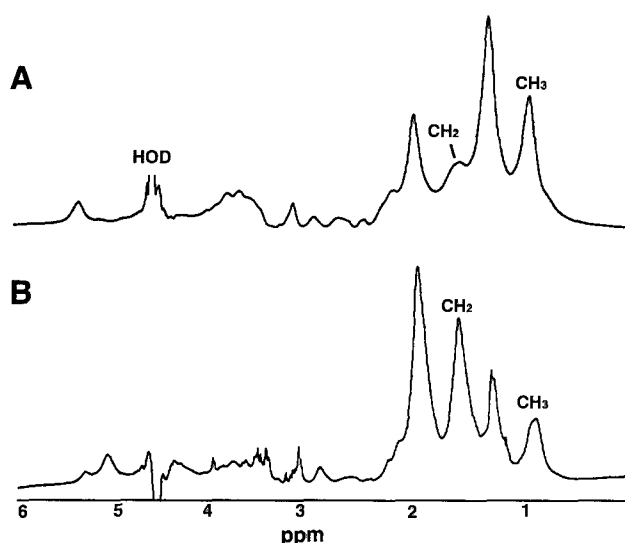


Figure 3. Proton magnetic resonance spectroscopy one-dimensional spectra from specimens of **A**, benign thyroid follicular adenoma, and **B**, follicular thyroid carcinoma. Diagnostic resonances at 0.9 ppm (CH_3) and 1.7 ppm (CH_2) are denoted. HOD = residual water. Reprinted with permission from Elsevier Science.³

proven carcinoma with a sensitivity and specificity of 100% ($P < 0.0001$, t test).⁸ Examples of one-dimensional spectra from follicular adenoma and follicular carcinoma are shown in Figure 3. The basis of the discrimination was altered cellular chemistry, which was reflected in the resonance-intensity ratio at a chemical shift of 1.7 ppm, arising from lysine, and lipid at 0.9 ppm. The lipid spectral profile is weaker in adenoma than carcinoma, and shows the same trend that was previously observed in uterine cervical cells.⁴

Follicular thyroid neoplasms not overtly malignant by current pathologic criteria spanned the normal and malignant spectral patterns (Figure 4). The biologic significance of the span of MRS ratios recorded for follicular lesions is unclear; however, it would support the concept

that there is a progression from benign to malignant cellular chemistry that precedes any histologic evidence of malignancy. Molecular genetic studies have recently supported this concept.²³

Fine Needle Aspiration Biopsy

Cytological examination of specimens taken from thyroid nodules by FNAB has been one of the most significant advances in the investigation of thyroid disease during the past decade. Unfortunately, there remains an even greater limitation to the technique than in histologic assessment; namely, the inability of the fine needle cytology to accurately discriminate between benign follicular lesions and follicular carcinomas.²⁴ Cytologic techniques are limited to the assessment of cellular characteristics, and can not determine whether follicular cells have penetrated the thyroid capsule or have invaded blood vessels, which are the two principal histologic determinants of follicular thyroid cancer. MRS of FNAB specimens can more accurately reflect the biology of follicular thyroid tumors and can allow a more definitive exclusion of the diagnosis of thyroid cancer, thereby reducing the need for unnecessary surgery performed solely for diagnostic purposes. It is possible to obtain an accurate proton magnetic resonance spectra from as few as 10^6 thyroid cells obtained during a FNAB.⁹

In a study of FNAB specimens and tissue specimens from 70 patients undergoing thyroidectomy for solitary or dominant thyroid nodules, a close correlation was found between fine needle magnetic resonance spectra and tissue spectra for a range of benign and malignant neoplasms.⁹ The sensitivity or probability of correctly identifying thyroid cancer on the basis of MRS from FNAB specimens was 95%; however, for this technology to be useful in a clinical setting, new data on MRS of FNAB obtained before surgery from thyroid lesions must be collected.

Figure 4. Ratio of resonances at 1.7 ppm and 0.9 ppm for normal thyroid tissue, follicular neoplasms and malignant thyroid biopsies. Histologically proved follicular cancers all have a ratio < 1.1 , whereas benign follicular neoplasms are comparable with either normal or malignant tissue. A separation of ratios more than and less than 1.1 is observed with no overlap between normal and malignant tissue. Reprinted with permission from Springer-Verlag.⁵⁸

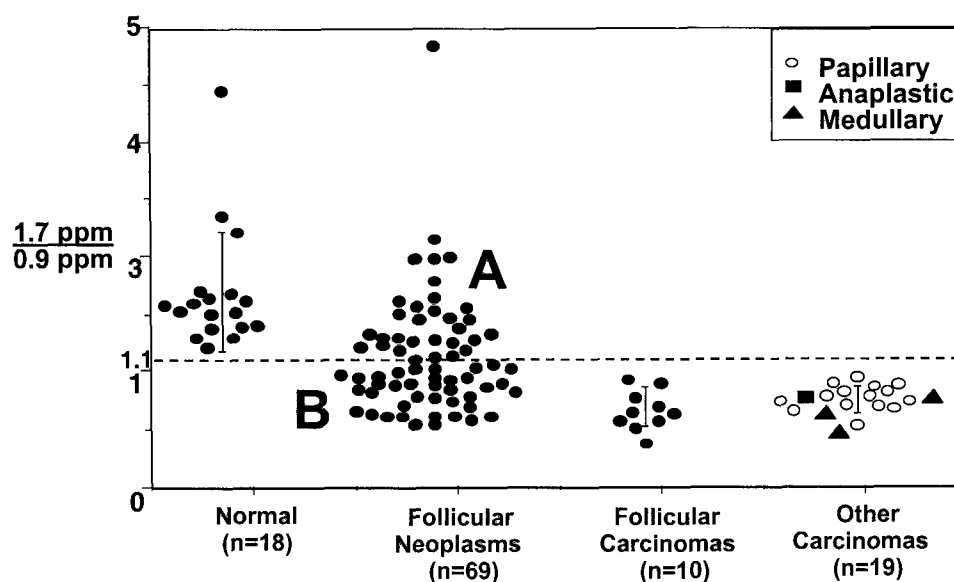
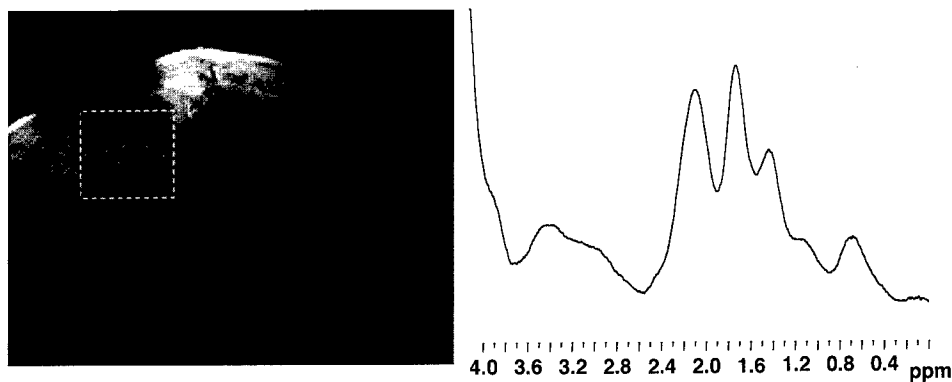


Figure 5. *In vivo* proton magnetic resonance spectrum of the thyroid at 3 T (performed at the National Research Council of Canada, Winnipeg). Data were obtained from a patient with a thyroid nodule using a specially designed multiring surface coil. **A**, T1-weighted axial image. **B**, Corresponding *in vivo* proton magnetic resonance spectroscopy spectrum. Reprinted with permission from King et al (King SB, Smith ICP, Tomanek B, unpublished data, 2000).



Statistical Classification Strategies

The resonances used to determine the pathology in the clinical studies described previously were identified visually, and resonance intensities were calculated manually and referenced with a second resonance that was used as an internal standard. Although these resonances correlated with specific biologic and pathologic features, there are many other spectral differences not easily discerned by the human eye that may be indicative of specific disease entities. For this spectral information to be useful, it is necessary to mathematically assess the similarities in the MRS data within groups of different samples, and to classify these data after thorough examination.

The application of statistical classification strategies has allowed further refinement of the discriminatory potential of spectral analysis. This methodology was developed by Dr. Ray Somorjai et al (Institute for Biomedical Sciences, National Research Council of Canada).²⁹ The most common conventional method is principal component analysis,²⁵ which is used for preliminary characterization of the data sets, particularly for data reduction. Optimal region-selection methods are also required, which retain spectral information that is essential for subsequent biochemical classifications. Independent analyses of different regions of the spectra are then undertaken. The analyses are typically performed using several types of classifiers, including neural nets,²⁵ linear and quadratic discriminant analysis,²⁶ and genetic programming.²⁷ These methods have proven superior to other approaches to the analysis of MRS data for cervical tumors.²⁸ The results of the various methods used to analyze the spectral regions are combined in a consensus analysis, which increases the classification power of the process significantly.

For thyroid cancer, no individual classification method (e.g., linear discriminant analysis, genetic programming, neural nets) was capable of high classification accuracy; therefore, a metaclassifier was developed that used the outputs of the individual classifiers as inputs to another classifier. This method led to a higher accuracy than any individual classification process.²⁹ When these data are subjected to statistical classification strategies, the training set has a sensitivity and specificity of 100%, and the test set of samples of known malignancy has a

sensitivity and specificity of 98% and 100%, respectively.²⁹

Spectroscopy In Vivo

In vivo spectroscopy of the thyroid at 3 T was undertaken at the National Research Council of Canada, Winnipeg. Typical data (Figure 5) were obtained from a patient with a thyroid nodule using a specially designed multiring surface coil. The spectroscopic data showed the lesion to be a benign adenoma on the basis of the *ex vivo* MRS studies, a diagnosis that was confirmed by histopathology (King SB, Smith ICP, Tomanek B, unpublished data, 2000).

As in cervical cancer, currently available *in vivo* thyroid data are preliminary but promising. Used in combination with the *ex vivo* thyroid data and diagnostic algorithms developed using computerized consensus diagnosis, proton MRS has the potential to provide a definitive, noninvasive diagnosis of thyroid nodules and to eliminate the need for surgery that is performed for nodule management and lesion diagnosis (i.e., to exclude malignancy).

Breast Cancer

Breast cancer is the most common cancer to affect women in Western countries. In Australia, its incidence outranks that of all other cancers in women older than 35 years. During the past decade, the incidence of breast cancer in Australia has risen by 25%, and the lifetime risk (i.e., 0–74 years) of breast cancer development in white Australian women is comparable to that of women in Western cultures, at approximately 7% to 8%.

Recent improvement in outcome of patients with breast cancer is due to earlier diagnosis through screening programs and more sophisticated management protocols.^{30,31} A combination of physical examination, mammography, and fine needle aspiration cytology or needle core biopsy—triple assessment—is currently the most sensitive method for the preoperative diagnosis of clinically and radiographically detected breast lesions. Although triple assessment has a high probability of detecting all malignant lesions, its suboptimal specificity results in diagnostic uncertainty, requiring open biopsy to exclude malignancy in many women.

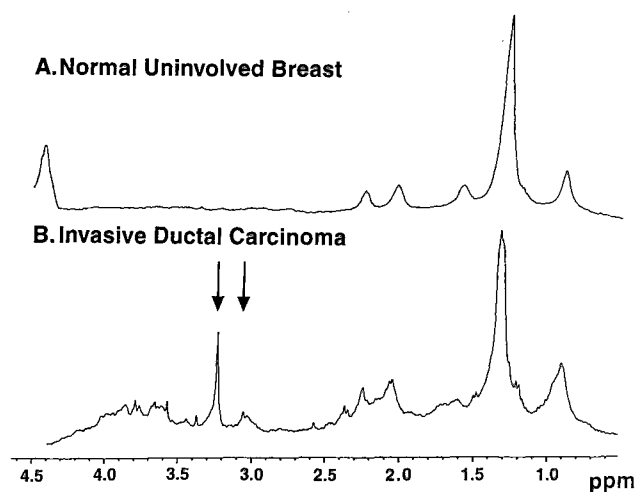


Figure 6. One-dimensional proton magnetic resonance spectra (256 acquisitions) of fine needle biopsy specimens obtained from a patient who underwent a mastectomy. Spectra for **A**, healthy, uninvolved breast tissue and **B**, invasive ductal carcinoma. The distinction between normal breast tissue and invasive carcinoma is based on an increase in the N-trimethyl resonance at 3.25 ppm normalized to that of creatine at 3.05 ppm (peaks indicated by arrows). Reprinted with permission from the Radiological Society of North America.⁷

Physical examination has limitations due to variation in breast consistency, the site and size of the lesion, and the presence of a diffuse *versus* discrete tumor. Screening mammography alone results in one benign lesion undergoing biopsy for every malignant lesion detected.³² However, 10% to 40% of palpable cancers are not detected using this modality, especially in women younger than 50 years in whom radiographically dense breast tissue may obscure changes associated with cancer.^{33–40} FNAB has a complete sensitivity of 81% to 97%⁴¹; however, this sensitivity includes atypical and suspicious diagnoses that are confirmed to be malignant after open biopsy in only 50% to 80% of cases.⁴² Although stereotactic needle core biopsy (SNCB) is increasingly used in place of FNAB in many countries, and with increased accuracy,⁴³ the procedure is associated with less than 100% accuracy.^{44, 45} Furthermore, SNCB is associated with an increased risk of complications and significantly reduced patient tolerance.⁴⁶ MRS suited to FNAB *versus* SNCB offers the accuracy of SNCB with the patient acceptance and low complication rate of FNAB. Further, SNCB is the preferred test in some countries; however, the procedure can not provide tissue samples that can be used in *ex vivo* MRS because of the high fat content in the intact tissue. The FNAB procedure mechanically separates the fat from the cells.

Spectroscopy Ex Vivo

In a study of 218 FNAB specimens from 191 consecutive patients undergoing diagnostic biopsy or definitive treatment (*i.e.*, lumpectomy, quadrantectomy, or mastectomy) for histologically proven invasive breast cancer, proton MRS identified invasive carcinoma by resonances

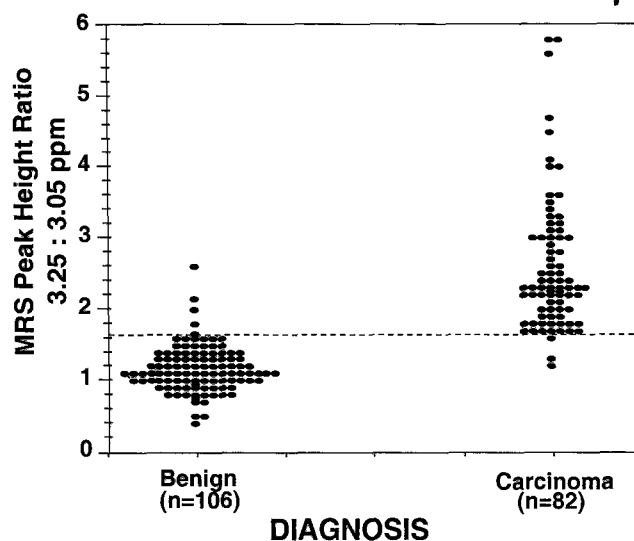


Figure 7. Breast fine needle biopsy magnetic resonance spectroscopic findings of unequivocally benign *versus* infiltrating carcinoma. Data are grouped on the basis of the final histopathologic findings in tissue specimens from the aspiration site. Reprinted with permission from the Radiological Society of North America.⁷

at 3.25 ppm, which were attributable to choline-containing metabolites (Figure 6).⁷ A discrimination between invasive carcinoma ($n = 82$) and normal or benign tissue ($n = 106$) was based on the intensity of the 3.25 ppm resonance standardized to the resonance intensity at 3.05 ppm, which contained contributions from creatine, phosphocreatine, and lysine ($P < 0.0001$, Mann-Whitney test) (Figure 7).⁷ Of 106 benign samples, 102 samples had a 3.25:3.05 ppm intensity ratio of less than 1.7 ppm. Four false-positive results were obtained from three palpable fibroadenomas and one lesion displaying moderate ductal hyperplasia.

Fine needle aspiration biopsy specimens from 4 of 82 malignant lesions had a magnetic resonance ratio of < 1.7 ppm. Histopathology of the aspiration site showed that one sample had only benign fibrocystic changes in this region; the three remaining samples were confirmed as invasive carcinoma with a marked inflammatory cell infiltrate.⁷ A number of specimens reported as DCIS by routine histopathology were assessed by MRS. Lesions containing only DCIS (10 high grade, one low grade) had magnetic resonance ratios of ≤ 1.7 ppm, which were indicative of a low choline to creatine/lysine ratio similar to that obtained for benign lesions. Four DCIS cases in which ductal cells had breached the basement membrane (< 1 mm) in one or more foci (*i.e.*, microinvasion) and two samples of high-grade DCIS with extensive comedonecrosis had magnetic resonance ratios of > 1.7 ppm, which were similar to those obtained for malignant lesions.⁷

By correlating the data from benign and malignant FNAB specimens with corresponding clinical data, all cases presenting as mammographically positive ($n = 56$) or mammographically negative ($n = 23$), and all cases regarded as nondiagnostic ($n = 14$) or atypical or suspi-

cious ($n = 25$) on FNAB, were correctly categorized by MRS as benign or malignant when compared with the histopathologic diagnosis of tissue excised from the aspiration site. MRS of FNAB specimens correlated with the final histologic diagnosis in 96% of benign lesions. This 4% false-positive rate compares favorably with the rate of other modalities when one considers that biopsy was performed because of clinical features (34%) or mammographic features (45%), or on FNAB cytologic indications (31%). Further, MRS of FNAB specimens correlated with a malignant histologic diagnosis in 95% of cases. No single preoperative modality improved the ability of MRS to correctly identify malignancy (physical examination, 84%; mammography, 82%; FNAB, 92%).⁷ The application of statistical classification strategies awaits sufficient numbers.

These data support the use of MRS of *ex vivo* FNAB specimens as a complementary procedure to the triple assessment regime. By providing an accurate and objective diagnosis of breast lesions, proton MRS *ex vivo* can greatly reduce the need for unnecessary open biopsy of benign lesions while reducing the anxiety due to equivocal diagnosis.

Spectroscopy In Vivo

In vivo proton MRS of the breast has the potential to improve staging and grading of breast cancer without recourse to surgery. By correctly separating invasive carcinomas from benign breast lesions, *in vivo* MRS will prevent up to 30% of excision biopsy procedures (*i.e.*, lumpectomies) currently performed to establish a diagnosis.

The Lenkinski laboratory⁴⁷ first demonstrated that benign and malignant breast lesions can be distinguished on the basis of differences in cellular chemistry using *in vivo* MRS at 1.5 T. Using localized proton MRS spectra obtained using a stimulated echo acquisition mode sequence of benign breast tissue ($n = 7$) and malignant breast tissue ($n = 10$) from clinical masses, increased choline metabolite levels were found in malignant tissue. However, the high and variable levels of fat tissue in the breast made it difficult to categorically state that metabolites indicative of cancer were absent from healthy tissue. More recently, Gribbestad et al⁴⁸ used image-guided localized proton MRS at 1.5 T on breast tissue and further substantiated choline to be increased in malignant lesions. Using a purpose-built double-breast coil and a point-resolved spectroscopy pulse sequence, Gribbestad et al⁴⁸ studied 22 subjects (10 healthy volunteers, 12 patients with breast cancer). Magnetic resonance spectra healthy breast tissue (Figure 8) showed resonances from water and lipid only. In spectra of malignant breast tissue (*i.e.*, ductal and undifferentiated carcinomas), a resonance at 3.3 ppm consistent with choline metabolites was also present (Figure 9). The results of these studies provided correct diagnoses; the data were obtained from large tumors (1–4 cm in diameter). It is clinically imper-

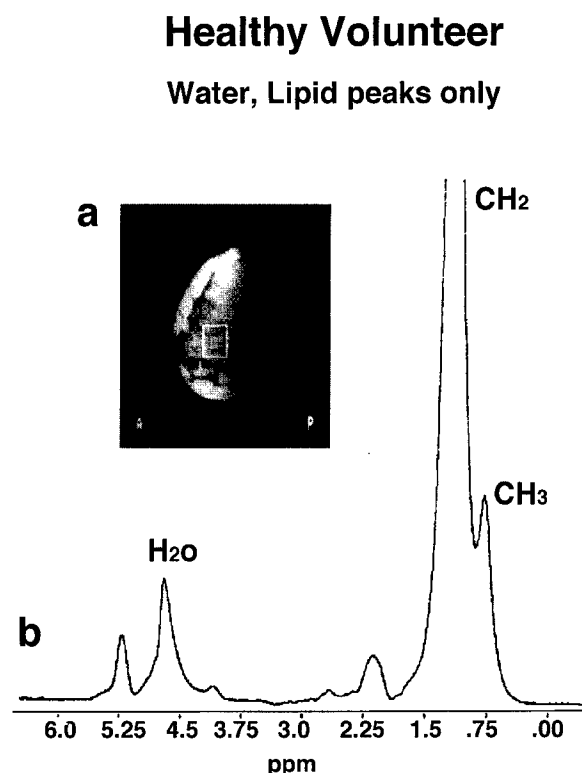


Figure 8. **A**, Sagittal magnetic resonance imaging (1.5 T) of a 47-year-old volunteer with a $20 \times 20 \times 20 \text{ mm}^3$ volume of interest localized within fatty breast tissue and **B**, the proton magnetic resonance spectrum ($TR = 2.000$, $TE = 136$, 256 averages) of the localized volume. Resonance signals are caused by water (4.8 ppm), terminal methyl (1.0 ppm), acyl chain methylene (1.3 ppm), α -methylene and β -methylene (2.3 ppm), and olefinic protons (5.4 ppm). Reprinted with permission from John Wiley and Sons.⁴⁸

ative to develop the method further, so that patients with smaller lesions can be investigated.

The large but variable fat content in breast tissue poses a significant problem in the use of MRS for *in vivo* detection and diagnosis. This problem is complicated by the levels of fat in breast tissue measurable by MRS that are modulated during the menstrual cycle. Three possible solutions to this problem are to (1) reduce the spectral contribution from the fat; (2) reduce the size of the voxel from which the spectra are obtained; or (3) observe a nucleus other than hydrogen.

Gribbestad et al⁴⁹ attempted the first solution, and demonstrated that the fat contribution to the *in vivo* proton MR spectrum may be reduced using a PRESS sequence with an echo time of 350 msec. Under these conditions, signals from choline and creatine are observed, and differences in the intensities of these resonances allow the differentiation of normal, lactating, abnormal benign, and malignant breast tissue.⁴⁹ Healthy breast tissue contains resonances from lipid only; however, healthy lactating breast tissue generates signals from lipid and creatine–choline, with a resonance at 3.5 ppm to 4.0 ppm. Only a small contribution from the choline–creatine resonance is observed in spectra of abnormal but benign breast tissue. The spectrum of malig-

Ductal Carcinoma

Choline peak at 3.3ppm

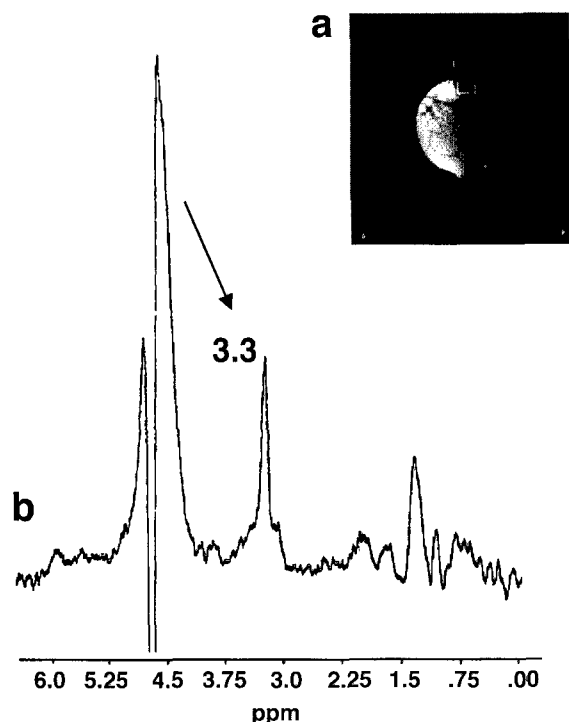


Figure 9. **A**, Sagittal magnetic resonance (1.5 T) of a 65-year-old patient with a ductal carcinoma and **B**, proton magnetic resonance spectrum of a 20 x 20 x 20 mm³ voxel (256 averages), showing the intense signal at 3.3 ppm. Reprinted with permission from John Wiley and Sons.⁴⁸

nant breast tissue contains dominant signals from both lipid and creatine–choline with no resonance at 3.5 ppm to 4.0 ppm.

Another means of reducing the signal from lipid was developed by Scott K et al (Scott K, Kozlowski P, Saunders J and Mountford C, unpublished data, 2000). Modification of the van Vaals pulse sequence,⁵⁰ including a further 180° pulse, has resulted in the acquiring combined echo pulse sequence that allows suppression of the lipid and water signals. This sequence, in combination with a breast-coil purpose built at the Institute for Biomedicine (National Research Council of Canada) allows choline and creatine diagnostic markers to be inspected in breast tissues *in vivo* in less than 20 minutes. The hardware and pulse sequence have not, however, been tested on women with known breast disease.

Phosphorus Spectroscopy *In Vivo*. Another means of solving the problem of high lipid content in breast tissue is to observe a nucleus other than hydrogen. Doyle et al⁵¹ have used three-dimensional chemical shift imaging (CSI) to acquire phosphorus (³¹P) spectroscopic data sets from patients with primary breast cancer. The use of CSI, which allows spectra to be collected from tumor tissue

and healthy tissue from the same breast during one experiment, was considered an advantage for confirming tumor-associated magnetic resonance changes in the breast because the ³¹P spectrum of healthy breast tissue can vary with age, menopausal status, stage of menstrual cycle, and lactation.

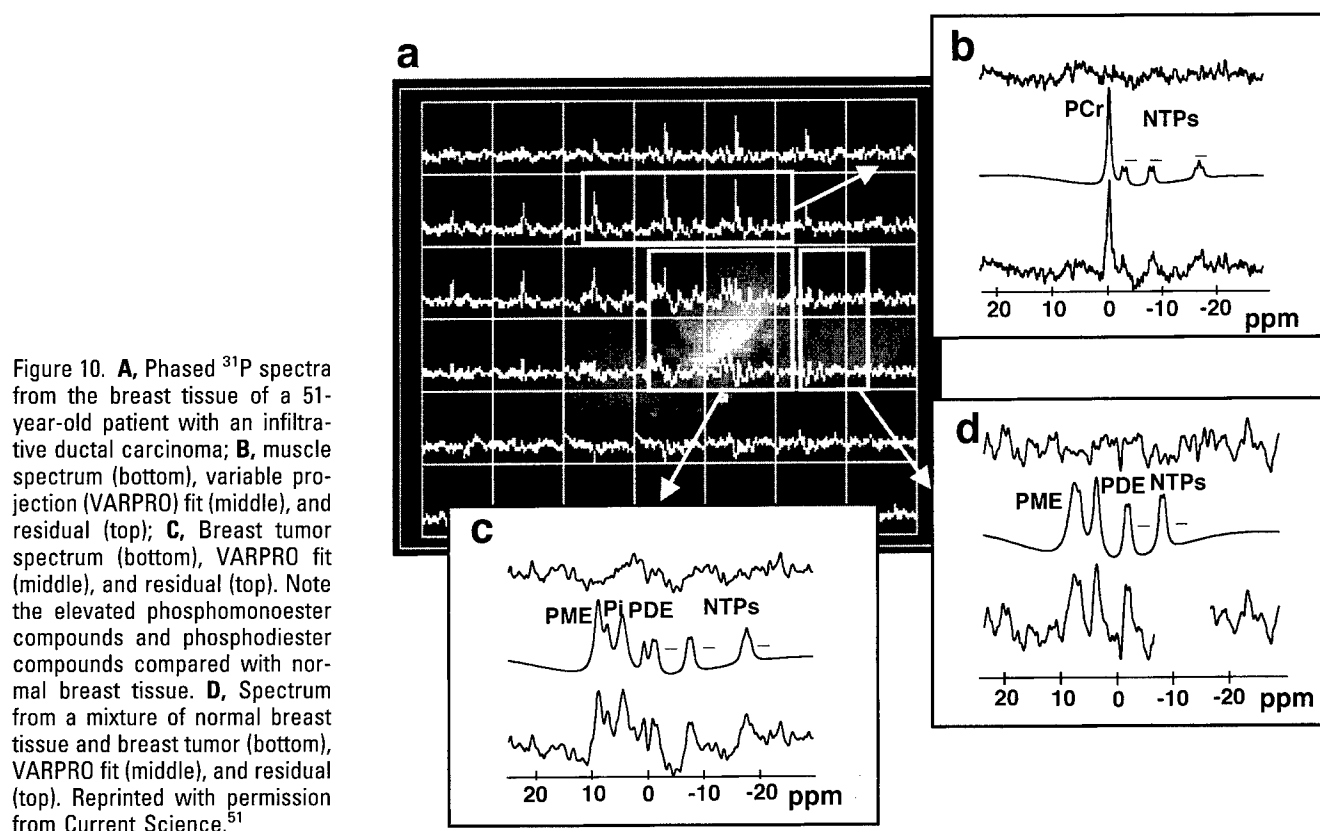
The central slice from a three-dimensional ³¹P CSI data set acquired at 1.5 T from a 51-year-old patient with an infiltrating ductal carcinoma is shown in Figure 10. Muscle spectra from the normal chest wall (Figure 10B) is readily distinguished from tumor spectra (Figure 10C) by the presence of intense signals from phosphomonoester compounds, phosphodiester compounds, and inorganic phosphate relative to signals from nucleotide triphosphates in the malignant spectrum. No phosphocreatine is observed in breast tumors compared with adjacent chest wall muscle. These data form part of a multicenter trial of ³¹P magnetic resonance spectroscopy funded by the National Cancer Institute (Washington, DC) to see whether decreases in signals from phosphomonoester compounds can be used as a predictor of tumor response during chemotherapy treatment.

Lymph Node Metastases in Patients with Breast Cancer

Axillary node status is currently the most accurate prognostic indicator in the treatment of patients with breast cancer, because therapy relies on assessment of these lymph nodes.⁵² Because of the inaccuracy of clinical and radiologic assessment of the axillary nodes, practical clearance of all nodes may be required for optimum treatment.⁵³ An unfavorable consequence of this clearance is the associated morbidity for the 60% of women who do not have axillary metastases. Unnecessary axillary surgery could be avoided if the status of the axillary nodes could be more accurately assessed before radical lymphadenectomy, which is the principle behind assessment of the so-called sentinel node. The sentinel node is the first node to receive lymph from the area of the breast containing the primary tumor. In breast cancer, the presence or absence of metastases in the sentinel node is an accurate predictor of the status of the remainder of the axilla.⁵³ However, the technique can only become an integral part of optimum management procedures when accurate intraoperative assessment of sentinel nodes is possible.

Histopathology, the primary method currently used to identify metastatic disease in lymph nodes, is subject to significant sampling error,^{54–56} and is not suited for intraoperative frozen-section diagnosis to exclude metastatic disease. A retrospective study of 921 patients with breast cancer in whom lymph nodes were diagnosed to be cancer-free after routine histologic examination of paraffin-embedded nodal tissue found evidence of metastatic breast cancer in 9% of lymph nodes after further detailed, labor-intensive examination.⁵⁵ Frozen sectioning of specimens introduces more sampling error, and renders intraoperative sentinel node diagnosis too inaccurate for safe clinical use.⁵³

Infiltrating Ductal Carcinoma



Spectroscopy Ex Vivo. Proton MRS *ex vivo* detects malignant cells in lymph nodes with a greater sensitivity than routine histology.¹⁰ In a rat model, two-dimensional correlated spectroscopy has been used to detect micrometastases not apparent when the node was serial sectioned for pathologic examination. The relative concentrations of the chemical species, lactate, choline, and amino acids facilitated the diagnosis. The magnetic resonance diagnoses were confirmed by xenographing nodes onto hairless mice.¹⁰ The clinical applicability of two-dimensional magnetic resonance experiments is limited by the long acquisition times (more than 4 h). This problem was overcome by Cheng et al⁵⁷ using one-dimensional magic angle spinning MRS to improve spectral resolution that obtained diagnostic information in 20 min. Specifically, one-dimensional magic angle spinning MRS distinguished normal nodes ($n = 14$) and metastatic tumor-bearing nodes ($n = 50$) by differences in the normalized magnetic resonance signal intensities (*i.e.*, the absolute magnetic resonance peak height and weight of sample) from creatine-phosphocreatine-lysine ($P < 0.0032$), lactate ($P < 0.0004$), and glutamate-glutamine ($P < 0.0002$).

A pilot study by Lo et al (Lo W, Dowd S, Malycha P, Russell P, Mountford C, Lean C, unpublished data, 2000) demonstrated that proton one-dimensional magic

angle spinning MRS can distinguish tumor-bearing and reactive lymph nodes based on differences in the relative intensities of resonances arising from lipid, amino acids, choline metabolites, and creatine. Axillary nodes from patients with breast cancer that were clinically positive but diagnosed cancer-free on routine histopathology were divided on the basis of the magnetic resonance data into two categories; one consistent with reactive nodes, and the other by magnetic resonance criteria, tumor bearing.

The diagnostic information obtained using magic angle spinning MRS is also available using conventional MRS by analysis of FNAB specimens of human lymph node tissue. This sampling method reduces the contribution from fat, and allows resonances from diagnostic chemicals that are present in the tissue in lower concentrations to be observed.

Proton one-dimensional MRS of FNAB specimens from an entire sentinel node will provide accurate identification or exclusion of micrometastases within the time scale required for intraoperative use. If MRS of FNAB specimens can predict nodal involvement, clearance will not be the prime treatment choice for node-free patients. Patients with nodal involvement may choose to undergo sentinel node biopsy to determine the extent of spread—a decision to be made by the surgeon and the

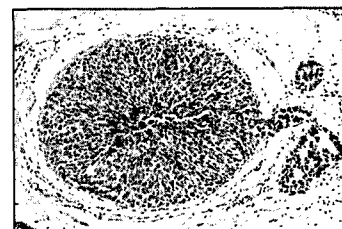
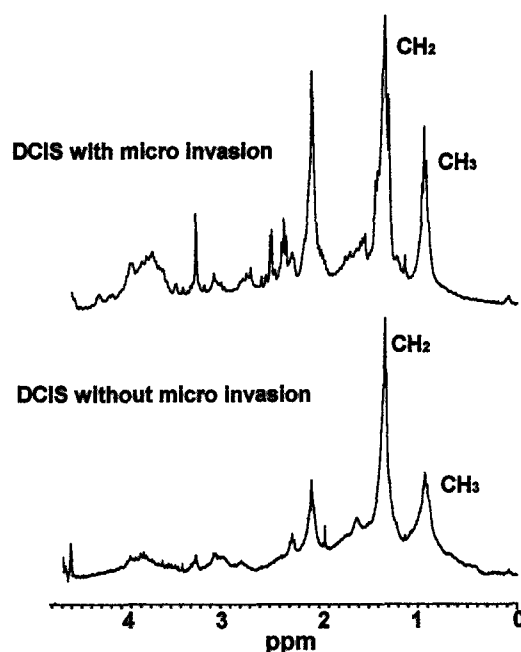


Figure 11. **A**, One-dimensional proton magnetic resonance spectra (256 acquisitions) of fine needle biopsy specimens obtained from breast ductal carcinoma *in situ* (top) with microinvasion present and (bottom) without microinvasion. **B**, Tissue sections of ductal carcinoma *in situ* taken 100 μ N apart (top) with microinvasion present and (bottom) without microinvasion.

patient. MRS will provide more definitive information for the treatment of patients who are genuinely node-free.

Magnetic Resonance Spectroscopy for Clinical Diagnosis

Despite an increasing number of successful studies that have used MRS to provide unique information that is clinically useful, the technique has been considered inadequate for clinical diagnosis. Three contributing factors were (1) sampling errors inherent in the biopsy process and histopathology; (2) difficulties with registration of MRS and pathologic data; and (3) the philosophy of spectroscopists.

It is inadequate to correlate routine histopathologic data with MRS data. The entire tissue specimen may need to be studied histologically at section intervals as small as 100 μ M. For example, in breast DCIS, which presents as a histologic continuum, epithelial cells change from premalignant cells to fully transformed malignant cells that are capable of invasion and tumor formation. Examining 100- μ M tissue sections enables the optimal assessment of this morphological continuum. The differences that may exist between two adjacent tissue sections 100 μ M apart is shown in Figure 11. The top section shows lobular involvement by DCIS with an obvious microinvasive component; however, the bottom section, which was obtained 100 μ M from tissue block, shows no evidence of invasion.

Inadequate registration of pathologic and MRS data has resulted in confusing and nonreproducible clinical MRS data. To obtain a correct pathologic correlation, it is important to register precisely from where the fine needle aspirate has been obtained or where the *in vivo* spectroscopy has been recorded. A biopsy specimen must then be taken from that precise area, and the paraffin

block step must be sectioned every 100 μ M for complete histopathologic assessment.

The prevailing philosophy among spectroscopists, who have historically been chemists or physicists, is that a sensitivity and specificity of 100% is unimportant if the biology and chemistry behind the diagnosis is not fully understood. This idea has contributed to the lack of clinical trials undertaken; however, as the field diversifies and clinicians and scientists work together more closely, this problem is being overcome.

The Future of Magnetic Resonance Spectroscopy in the Clinic

Spectroscopy of biopsy specimens has the potential to become the new gold standard for medical diagnosis. Used as an adjunct to conventional pathology, MRS can detect small populations of abnormal cells with high sensitivity and specificity. Of particular importance is the capacity of MRS to subcategorize preinvasive states. This capacity may prove the most valuable contribution of MRS, because recognition of preinvasive states with committed malignant potential is the great missing link in our diagnostic chain that allows optimum management and minimalist therapy of the many patients in whom cancer has not developed. However, before the *ex vivo* MRS method can be implemented as a routine clinical procedure in health care, the necessary equipment and quality controls need to be developed, the relevant mathematical algorithms for each organ and disease state need to be incorporated, and these elements must be tested in a multinational trial. These endeavors are underway. Finally, the use of MRS to analyze tissues that have been treated with radiotherapy or chemotherapy requires a new database, which must be collected and analyzed using MRS and statistical classification strategies.

Spectroscopy *in vivo* requires further development before it is introduced to clinical practice. The small cohort studies using spectroscopy *in vivo* have shown the method to be useful for the study of breast, thyroid, and cervical disease; however, smaller voxel sizes are required, particularly for breast diagnosis, if the method is to be useful. In addition, precise registration and correlation with histopathology is needed so that a computerized consensus diagnosis can provide a robust clinical diagnosis. For this exciting new application to be realized, clinical studies must include detailed pathologic assessment of all tissues analyzed by MRS. If MRS data are not compatible with histologic data, the entire tissue must be examined without knowledge of the MRS diagnosis, and the MRS data reviewed, to ensure that the discrepancy is not due to sampling error. These studies are tedious and time consuming, but they must be undertaken. The spectroscopy community needs to work closely with pathologists and surgeons to ensure that spectroscopy is introduced only after the appropriate rigorous testing procedures and clinical studies have been performed.

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